AN EVALUATION OF THE RELATIONSHIP BETWEEN PERIODONTAL DISEASE STATUS AND GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

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CERTIFICATE

This is to certify that **Dr. H. GAYATHRI,** Post Graduate student (2008 – 2011) in the Department of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai – 600 003, has done this dissertation titled '**AN EVALUATION OF THE RELATIONSHIP BETWEEN PERIODONTAL DISEASE STATUS AND GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS'** under our direct guidance and supervision in partial fulfillment of the regulations laid down by the **Tamil Nadu Dr. M.G.R. Medical University,** Chennai – 600 032 for **M.D.S.,** (Branch II) **Periodontology** degree examination.

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ABSTRACT

Background: Periodontal disease status in diabetic patients has been shown to correlate positively with Plasma HbA1c. But its association with serum Fructosamine remains unclear, more so, when the severity is expressed as a continuous quantitative variable.

Aim: To analyze the relationship between HbA1c, Fructosamine (FA) and periodontal disease severity expressed as Attachment Loss Surface Area (ALSA) and Periodontal Inflamed Surface Area (PISA) in patients with and without Type 2 Diabetes Mellitus.

Materials and Methods: ALSA and PISA were calculated in 60 type 2 diabetics and 40 non-diabetics with generalized chronic periodontitis and their HbA1c and FA levels were assessed using an enzymatic assay. Pearson correlation was used to analyze the association between these variables. Multiple linear regression (backward) was performed to analyze the predictability of ALSA and PISA from these glycemic markers, age, gender, duration of diabetes and Plaque index (Pl I).

Results: The glycemic markers showed a very high inter-correlation in the diabetics and a moderate correlation in the non-diabetic group. They exhibited a good to moderate correlation with ALSA and PISA in the diabetics and none significant in the non-diabetics. Multiple regression analysis revealed that the most significant predictors for ALSA in the diabetic group were the glycemic control, Pl I and age, while those for PISA were glycemic control and Pl I. The only significant predictor of PISA in the non-diabetics was Pl I and none was significantly predictive of ALSA in this group.

Conclusions: Fructosamine is a valid alternative to HbA1c for the evaluation of glycemic status in generalized chronic periodontitis patients with Type 2 Diabetes. Poor glycemic control has a significant and direct contribution towards ALSA and PISA in these patients.

DECLARATION

TITLE OF DISSERTATION	**"An evaluation of the relationship between periodontal disease status and glycemic control in patients with type 2 diabetes mellitus"**
PLACE OF STUDY	Tamil Nadu Government Dental College & Hospital, Chennai-600003
DURATION OF THE COURSE	3 Years
NAME OF THE GUIDE	Dr. S. KALAIVANI
HEAD OF THE DEPARTMENT	Dr. K.MALATHI

I hereby declare that no part of the dissertation will be utilized for gaining financial assistance/any promotion without obtaining prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai -600003. In addition, I declare that no part of this work will be published either in print or in electronic media without the guide who has been actively involved in dissertation. The author has the right to reserve for publish of work solely with the prior permission of the Principal, Tamil Nadu Government Dental College & Hospital, Chennai-600003.

Head of the Department **Guide** **Signature of the candidate**

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LIST OF ABBREVIATIONS

µL	Microlitre
µmol/L	Micromoles/Litre
AGE	Advanced Glycation End Products
AL	Attachment Loss
ALSA	Attachment Loss Surface Area
BCDM	Better Controlled Diabetes Mellitus
BMI	Body Mass Index
BOP	Bleeding on Probing
CAL	Clinical Attachment Level
CEJ	Cemento-Enamel Junction
CIDD	Controlled Insulin Dependent Diabetes Mellitus
DCCT	Diabetes Control and Clinical Trials
DM	Diabetes Mellitus
FA	Fructosamine
GCF	Gingival Crevicular Fluid
GHb	Glycated Hemoglobin
GI	Gingival Index
GSP	Glycated Serum Protein
HbA1c	Hemoglobin A1c
HPLC	High Performance Liquid Chromatography
IDDM	Insulin Dependent Diabetes Mellitus

LGM	Location of Gingival Margin
NBT	Nitroblue Tetrazolium
NIDDM	Non Insulin Dependent Diabetes Mellitus
OGTT	Oral Glucose Tolerance Test
OPD	Out Patient Department
PASW	Predictive Analytics Software
PCDM	Poorly Controlled Diabetes Mellitus
PD	Probing Depth
PESA	Periodontal Epithelial Surface Area
PIDD	Poorly Controlled Insulin Dependent Diabetes
PISA	Periodontal Inflamed Surface Area
Pl I	Plaque Index
PPD	Probing Pocket Depth
R1a, R1b, R2	Reagent 1a, 1b, 2
RR	Relative Risk
RSA	Recession Surface Area
SPSS	Statistical Package for Social Sciences
T2D	Type 2 Diabetes
TBA	2-Thiobarbituric Acid
THb	Total Hemoglobin

INTRODUCTION

Periodontal disease. The Sixth Complication of Diabetes Mellitus

- Loe H (1993)[1]

Diabetes mellitus is the second most common metabolic disorder in human with Type 2 diabetes (formerly Non Insulin Dependent Diabetes Mellitus) accounting for 90-95% of all the cases.[2] Chronic periodontitis is defined as "an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss."[3] Though there is an essential bacterial etiology, the interaction of the micro-organism with the host determines the course and extent of the resulting disease. Several factors like systemic disorders and conditions, environmental, physical and psychosocial factors have the potential to alter the periodontal tissues and the host immune response, resulting in more severe periodontal disease expression.[4]

Diabetes is a clear risk factor for periodontitis.[5] Hence the evaluation of the glycemic status of a patient is mandatory prior to any periodontal intervention. The traditional methods like random blood glucose, fasting blood glucose and two hour post-prandial glucose offer only the "Snapshot" information, i.e. glucose concentration at that point of time, and are of limited value from a periodontal point of view.[4] HbA1c has been considered the gold standard for the evaluation of the long term glycemic status in a diabetic patient.[6,7] Fructosamine assay, the alternative to HbA1c has shown good correlation with HbA1c[8,9] with additional advantages of being economical and simple in laboratory procedure.[9] Further, it serves as a valuable alternative in conditions where HbA1c is likely to be misleading such as hemoglobinopathies or anemia.[8]

The severity of the periodontal disease is often represented as non-continuous variables (mild, moderate, severe etc) which in the truest sense do not quantify the

amount of affected periodontal tissue. Using continuous variables such as mean probing pocket depth or mean clinical attachment level has partly addressed this issue, but the primary problem still remains.[10,11] A classification that quantified the total surface area of attachment loss, later referred to as Attachment Loss Surface Area (ALSA) was given by Hujoel et al.[12] Another important parameter, the Periodontal Inflamed surface Area (PISA) which quantifies the systemic burden of the periodontal disease in a patient was proposed by Nesse et al.[10]

Though the literature is replete with the studies on the association between periodontal disease status and Plasma HbA1c, there is a dearth of literature regarding its relationship with serum fructosamine level. The paucity is even more pronounced for the comparative evaluation of the association between these two measures of Glycemic control and the periodontal disease severity quantified in terms of ALSA or PISA. Therefore, it was deemed appropriate to conduct a study to comparatively analyze the relationship between plasma Glycated hemoglobin and serum Fructosamine levels and the severity of Generalized Chronic Periodontitis expressed in terms of ALSA and PISA in patients with and without Type 2 Diabetes mellitus.

AIM AND OBJECTIVES

1. To evaluate the association, if any, between plasma Glycated Hemoglobin level and periodontal disease severity expressed as Attachment Loss Surface Area (ALSA) and Periodontal Inflamed Surface Area (PISA), in generalized chronic periodontitis patients with and without Type 2 Diabetes mellitus.

2. To assess the association, if any, between serum Fructosamine level and periodontal disease severity expressed as ALSA and PISA, in generalized chronic periodontitis patients with and without Type 2 Diabetes mellitus.

3. To evaluate the predictability of the periodontal disease severity from these two measures of glycemic control in generalized chronic periodontitis patients with and without Type 2 Diabetes mellitus.

REVIEW OF LITERATURE

Historical perspective of HbA1c and Fructosamine

HbA1c

Hemoglobin A1c was first separated from other forms of hemoglobin by **Huisman** and **Meyering** in 1958 [13] using a chromatographic column.

HBA1c was characterized as a glycoprotein by **Bookchin and Gallop** in 1968.[14]

Its increase in diabetes was first described in 1969 by **Rahbar S et al.**[15] An unusual hemoglobin was found in patients with diabetes mellitus which resembled those of hemoglobin A_{1c} prepared from normal subjects. They suggested the possibility that an amino sugar is bound to hemoglobin A_{1c} in diabetic patients.

The reactions leading to the formation of HbA1c were characterized by **Bunn HF** and his co-workers in 1975. [16] They proposed that in the red cell, glucose binds to the α-amino position of hemoglobin β-chains (valine) in an aldimine (Schiff base) linkage. This aldimine can then partially rearrange in a reversible manner to form a ketoamine linkage which is stable to acid hydrolysis. This Amadori-type of rearrangement accounts for the formation of mannose, the C-2 epimer of glucose, as well as the inability to demonstrate ^{3}H---Na BH_4 reduction at the C-1 position.

H F Bunn et al [17] stated that HbA1c is formed by the condensation of glucose with the N-terminal amino groups of the beta-chains of Hb A. The specific activity of Hb A1c was found to be significantly lower than that of Hb A, suggesting that the formation of Hb A1c is a posttranslational modification. Its specific activity rose slowly, reaching that of Hb A by about day 60, indicating that Hb A1c is formed during the 120-day life-

span of the erythrocyte, probably by a non enzymatic process. Patients with shortened erythrocyte life-span due to hemolysis had markedly decreased levels of Hb A1c.

Koenig RJ [18] studied the increased levels of hemoglobins A_{Ia+Ib} and A_{Ic} in five hospitalized diabetic patients to determine whether changes in diabetic control would cause parallel changes in the levels of these hemoglobins.. The**y** concluded that periodic monitoring of hemoglobin A_{Ic} levels provided a useful way of documenting the degree of control of glucose metabolism in diabetic patients and served as a means to assess the relation of carbohydrate control to the development of sequelae.

Nathan DM [19] evaluated the clinical information value of the glycosylated hemoglobin assay by comparing it with practitioners' estimates of glucose control over the preceding 10 weeks in 216 diabetic patients. They concluded that the glycosylated hemoglobin assay provided information about the degree of long-term glucose control that is not otherwise obtainable in the usual clinical setting.

John WG et al [20] described a method for estimating hemoglobin A1c **(**HbA1c**)** with a commercially available enzyme immunoassay system. The method was based on micro titer plate technology, utilizing an antibody raised to hemoglobin**,** the epitope being the Amadori product of glucose plus the first eight amino acids on the N-terminal end of the beta chain of hemoglobin. Using this method, they obtained a reference interval of 2.8-4.9% (central 95%) for HbA1c in a non-diabetic population. The percentages of hemoglobin that were HbA1c in diabetics (6.86% +/- 2.51%) were significantly greater than in non-diabetics (3.46% +/- 0.52%)**.**

Liu et al [21] developed and validated a direct enzymatic HbA1c assay that utilized a single channel on chemistry auto-analyzers without the need to run separate glycated hemoglobin and total hemoglobin assays. Diazyme Direct Enzymatic HbA1c Assay was contended to be accurate and precise when compared to currently marketed medical devices. The method was not adversely affected by interferences from common hemoglobin variants in samples and considered to be cost effective, user-friendly and adaptable to most general chemistry analyzers.

Fructosamine

Johnson RN [22] developed a novel manual method to measure serum glycosylprotein as an index of diabetic control. The method relied on the ability of ketoamines (fructosamines) to act as reducing agents in alkaline solution. The simple colorimetric procedure permitted the assay of both a synthetic fructosamine and purified albumin while severely limiting the contribution of interfering substances.

Armbruster DA[8] reviewed five different methodologies to measure fructosamine: Phenylhydrazine procedure, Furosine procedure, Affinity chromatography, 2-thiobarbituric acid colorimetric (TBA) procedure and Nitroblue tetrazolium colorimetric (NBT) procedure. He stated that of the different methods, Affinity chromatography and the NBT methods appeared to be the most practical means to assess fructosamine quickly, economically and accurately. As fructosamine reflected the average blood sugar concentration over the past two to three weeks, it possessed a clinical advantage of responding more quickly to changes in therapy than HbA1c, thereby allowing improved glycemic control.

Baker et al [23] used fructosamine (FA) assay as a screening test for occult diabetes in groups of patients referred for oral glucose tolerance tests (OGTT) and found the specificity and sensitivity of fructosamine as a true diabetes predictor to be 88 and 91% respectively. The fructosamine assay's ability to distinguish between subjects with impaired glucose tolerance and those with true diabetes was a definite advantage of the test. The range of fructosamine, using the NBT assay was estimated to be 1.23 – 2.15 mmol/L in normal patients and 1.74 to 3.10 mmol/L in those diagnosed as diabetic.

Ludvigsen CW et al [24] found a very high degree of correlation (r = 0.8909) between the hemoglobin Alc and fructosamine measurements in 269 proposed insured whose blood glucose value was greater than 115 mg/dl. Using the fructosamine frequency graph compiled over a six-day period with 5000 specimens they found the upper limit (95th percentile) of normal to be 2.1 mmol/L.

Kouzuma T et al [25] developed an automated diazyme enzymatic assay for glycated albumin in blood samples. The method involves use of albumin-specific proteinase, ketoamine oxidase and serum albumin assay reagent. Glycated albumin detected by this method correlated significantly with that detected by high-performance liquid-chromatographic (HPLC) method. They concluded that the new enzymatic method was simple, rapid, allowed multiple determinations and enabled quantitative analysis of glycated albumin.

Wang et al [26] developed an enzymatic assay (The Diazyme GSP enzymatic assay) for the in-vitro quantification of serum GSP. The assay demonstrated good correlation with the Randox Fructosamine assay and was not affected by ascorbic acid,

bilirubin, hemoglobin, glucose, triglycerides, or uric acid at concentrations commonly found in patient samples. They also developed applications of this assay for commonly used automated chemistry analyzers.

Relationship between glycemic control and periodontal status

Sastrowijoto SH et al [27] evaluated the relationship between glycemic control and the periodontal status in 22 type 1 diabetic adults. Patients were studied in two groups: near normal metabolic control (HbA1c $\leq$ 7.7%) and those with poor metabolic control (HbA1c $\geq$ 9.9%). No significant difference was found between the 2 test groups with regard to periodontal condition and neither age nor the duration of diabetes mellitus influenced the periodontal parameters.

Piché JE, Swan RH and **Hallmon WW** [28] presented two case reports to illustrate how the glycosylated hemoglobin assay can be utilized by the periodontists. This assay was presented as a relatively new test used in the diagnosis and monitoring of the diabetic patients. Indication of the blood glucose level over an extended period of time (30 to 90 days) and no requirement for fasting prior to testing were considered as the main advantages of this method over the traditional assays.

Sastrowijoto SH et al [29] evaluated the effect of improved metabolic control on the clinical periodontal condition and the sub-gingival microflora of diseased and healthy periodontal pockets in 6 ambulatory IDDM patients. HbAlc improved significantly with intensive conventional insulin treatment. No effect could be demonstrated for PPD, probing attachment level, BOP and the plaque index. Statistical analysis of improved metabolic control on the sub-gingival microflora revealed that the percentage of streptococci increased significantly only in diseased pockets. The results of the study

indicated that improved metabolic control in IDDM patients had no potential impetus for an improved clinical periodontal condition or on the sub-gingival bacterial flora.

Safkan-Seppälä B and **Ainamo J** [30] assessed the frequency and severity of periodontal disease in 44 individuals with poorly controlled IDDM (PIDD, mean blood glucose level of 11.8mmol/l and mean HbA1 level of 10.7%) and 27 subjects with controlled IDDM (CIDD). Site-specific recordings were made for the plaque index, gingival index, pocket depth, loss of attachment, bleeding after probing, gingival recession and radiographic loss of alveolar bone. Under similar plaque conditions, adult subjects with a long-term PIDD were found to have lost more approximal attachment and bone than subjects with a CIDD.

de Pommereau V et al [31] evaluated the periodontal status of 85 French adolescents with IDDM and 38 healthy controls in the same age group. Plaque control, gingival inflammation and probing attachment level were evaluated. The inter-proximal marginal bone level was assessed with bitewing radiographs taken on the first molars and on areas presenting an attachment loss over 2 mm. Diabetic children had significantly more gingival inflammation but no significant relation was found between gingival condition and age, Tanner's index, HbAlc level or disease duration. None of the subjects had sites with attachment loss $\geq$ 3 mm or radiographic signs of periodontitis.

Seppälä B, Seppälä M and **Ainamo J** [32] investigated the progression of periodontal disease in 26 subjects with poorly controlled IDDM (PIDD, mean blood glucose of 12.5 mmol/l and a mean HbA1 of 10.1%) and 12 subjects with controlled IDDM (CIDD, mean blood glucose of 6.7 mmol/l and mean HbA1 of 9.2%). The plaque index, gingival index, pocket depth, loss of attachment, bleeding after probing, gingival

recession, and radiographic loss of alveolar bone were recorded. At baseline and 2 years after the baseline examination, the PIDD subjects had similar plaque conditions, but had more gingivitis and bleeding after probing when compared to the CIDD subjects

Tervonen T and **Oliver RC** [33] evaluated the association between long-term control of diabetes mellitus (DM) using the mean of HbAlc over the past 2-5 years and periodontitis in 75 diabetics. Plaque, calculus (+/-), probing depth (pd) and attachment loss (al) were recorded in a randomized half-mouth examination. An increase in the prevalence, severity and extent of periodontitis with poorer control of diabetes was observed. In a multiple regression analysis, calculus and long-term control of diabetes were significant variables when pd $\geq$ 4 mm. With calculus, the frequency of pd $\geq$ 4 mm increased from 6% in the well-controlled diabetics to 16% in the poorly-controlled ones. They concluded that periodontitis in diabetics is associated with long-term metabolic control and presence of calculus.

Unal T et al [34] investigated the relationship between the diseased state of the periodontal tissues and serum fructosamine and the plasma glucose values in 71 non-insulin dependent diabetes mellitus (NIDDM) patients and 60 non-diabetics. There was a positive correlation between fructosamine and the degree of gingival bleeding (0.684), however serum glucose levels had little or no correlation.

Pinson M et al [35] compared the periodontal status of a juvenile diabetic study group with that of a non-diabetic controls. No statistically significant differences were found between the groups for average attachment loss, probing depths, recession, gingival index, plaque index, gingival fluid flow, bleeding on probing and there was no correlation between the level of diabetic control (Glycated Hb) and clinical variables.

However, the diabetic group had a higher average gingival index for most teeth and higher or the same plaque index levels on all teeth relative to controls. Thus, a young study population with type I diabetes mellitus was found to have significantly increased severity of inflammatory gingival disease compared to controls of similar age.

Novaes AB Jr, Gutierrez FG and **Novaes AB** [36] evaluated the periodontal disease progression of 30 Type 2diabetics and 30 non-diabetics. The diabetics were divided into three subgroups: controlled, moderately controlled, and poorly controlled at the end of the study. When comparing the two groups as a whole significant difference was observed for AL. When diabetic patients were divided into subgroups, significant differences were observed between the poorly controlled and the control groups for both the PPD and periodontal attachment loss. The glycosylated hemoglobin test was found to be more reliable than the fasting glucose analysis for the purpose.

Firatli E, Yilmaz O and **Onan U**[37] examined the periodontal status of 77 IDDM children and adolescents, 77 paired healthy, sex- and age-matched controls. Fasting blood glucose, fructosamine and HbA1 values were determined. The mean PPD, CAL and the parameters to assess diabetes mellitus were significantly higher for the diabetic group. They found a positive correlation between the duration of diabetes and clinical attachment loss and between the serum fructosamine and gingival index in the diabetic group.

Taylor GW et al [38] tested the hypothesis that severe periodontitis in persons with NIDDM increased the risk of poor glycemic control. Data from Gila River Indian Community were analyzed for dentate subjects aged 18 to 67, diagnosed at baseline with NIDDM baseline HbA1 < 9%; and who remained dentate during the 2-year follow-up.

Medical and dental examinations were conducted at 2-year intervals. Severe periodontitis was specified as baseline periodontal attachment loss of $\geq$ 6 mm on at least one index tooth; baseline radiographic bone loss of 50% or more on at least one tooth. Poor glycemic control was specified as HbA $\geq$ 9% at follow-up. Severe periodontitis at baseline was associated with increased risk of poor glycemic control at follow-up. Other statistically significant covariates were age, level of glycemic control, severe NIDDM, duration of NIDDM and smoking: at baseline. These results supported severe periodontitis as a risk factor for poor glycemic control.

Collin HL et al [39] investigated the periodontal status of 25 patients with (NIDDM) (age range 58 to 76) and 40 non-diabetics (age range 59 to 77). Surfaces with visible plaque and bleeding after probing, calculus, total AL and mean alveolar bone loss were evaluated. Periodontal disease was considered advanced when mean alveolar bone loss was over 50%, or 2 or more teeth had pockets $\geq$ 6 mm. Patients with NIDDM had significantly more advanced periodontitis than control subjects. The HbA1C level deteriorated only in patients with advanced periodontitis, when compared to the situation 2 to 3 years earlier. Therefore, advanced periodontitis was considered to be associated with the impairment of the metabolic control in patients with NIDDM.

Stewart JE et al [40] explored the effect of periodontal therapy on glycemic control in type 2 diabetics. 72 type2 DM patients were studied, of whom 36 received therapy for adult periodontitis and the rest without treatment formed the control group. During the nine-month observation period, control group had 6.7% improvement when compared to a 17.1% improvement in the treatment group, a statistically significant difference. They

interpreted the data to suggest that periodontal therapy was associated with improved glycemic control in persons with type 2 DM.

Alpagot T et al [41] investigated the associations between GCF elastase levels, clinical measures of periodontal status and metabolic control of diabetes (HbA1c) in type 1 and 2 diabetes. The results indicated that the elastase levels significantly correlated with periodontal parameters in both the groups. However HbA1c levels did not correlate with clinical measurements and GCF elastase. The results suggested that GCF elastase, age and smoking are risk indicators for periodontitis in patients with diabetes mellitus, and periodontal status is not associated with the duration and metabolic control of diabetes.

Tsai C, Hayes C and **Taylor GW** [42] investigated the association between glycemic control of type 2 diabetes mellitus and severe periodontal disease based on data from 4343 persons from US adult population. Severe periodontal disease was defined as 2+ sites with 6+ mm loss of attachment and at least one site with probing pocket depth of 5+ mm. Individuals with poorly controlled diabetes (PCDM) had glycosylated hemoglobin > 9% and those with better-controlled diabetes (BCDM) had glycosylated hemoglobin ≤ 9%. The data indicated that the individuals with PCDM had a significantly higher prevalence of severe periodontitis than those without Diabetes. For the BCDM subjects, there was a tendency for a higher prevalence of severe periodontitis. These results were considered to provide population-based evidence to support an association between poorly controlled type 2 diabetes mellitus and severe periodontitis.

Syrjälä AM et al [43] analyzed the role of smoking and HbA1c level in attachment loss (AL) and probing depths (PDs) among 64 IDDM patients. Data were obtained from

patient records and by clinical examination. The outcome variables were the number of sites with AL and PD of 5-9 mm. The results showed that the Relative risk (RR) among the smokers was 4.15 for AL and 7.96 for PD. HbA1c was not related to AL or PD. Among smokers with HbA1c > 8.5, RR for AL was 12.34. It was concluded that the poor metabolic control together with smoking is extremely detrimental for attachment loss.

Jansson H et al [44] analyzed the association between medical characteristics and severe periodontal disease in 191 subjects with type 2 diabetes (T2D). Based on assessment of marginal bone height in panoramic radiographs, two subgroups were identified: periodontally diseased and periodontally healthy group. Periodontally diseased (20% of the subjects) individuals had higher HbA1c levels and higher prevalence of cardiovascular complications. The best predictor for severe periodontal disease in subjects with T2D was smoking followed by HbA1c levels.

Nesse W et al [10] discussed the shortcomings of the existing classification methods for periodontitis that expressed the severity of the disease as non-continuous variables (mild, moderate severe etc.). A new method that quantitatively expressed the severity of periodontal disease using full mouth Clinical Attachment Level (CAL), recessions and bleeding on probing (BOP) measurements. Attachment Loss Surface Area (ALSA) was generated by transforming linear CAL measurements around a particular tooth into surface area for that particular tooth. Periodontal Epithelial Surface Area (PESA), the surface area of pocket epithelium, was calculated from ALSA and Recession Surface Area (RSA). Finally, the part of the PESA that is affected by BOP was termed the Periodontal Inflamed Surface Area (PISA). This parameter was presented as the main contributor to the systemic inflammatory burden posed by periodontitis and hence more

relevant in assessing the relationship of periodontal disease with any systemic disorders. Microsoft Excel spreadsheets that were specially designed to calculate all these parameters from BOP, PPD and CAL measurements were presented along with details about their online resources.

Nesse W et al [11] investigated whether a dose-response relationship existed between the Periodontal Inflamed Surface Area (PISA) and HbA1c levels in 40 dentate type 2 diabetics. PISA was calculated from full-mouth PPD and BOP measurements. HbA1c levels were retrieved from patient's medical files. Multiple linear regression analysis were performed to analyze the association between these two variables The results showed that the higher the PISA of type 2 diabetics, the higher their HbA1c levels. On a group level, an increase of PISA by 333 mm^2 was associated with a 1.0 % increase of HbA1c, independent of the influence of other factors. They concluded that, on a group level, there is a dose-response relationship between PISA and HbA1c in type 2 diabetics. This might be an indication of a causal relationship between type 2 diabetes and periodontitis.

Hayashida H et al [45] analyzed the relationship between periodontal status and glycosylated hemoglobin (HbA1c) in non-diabetic subjects. Periodontal status, HbA1c, serum cholesterol, triglyceride, body mass index (BMI), and demographic variables were assessed in 141 Japanese adults. The difference in the HbA1c level was evaluated among subjects according to periodontal status. The mean HbA1c was significantly elevated with periodontal deterioration. It was concluded that there was a significant relationship between periodontal status and HbA1c levels in non-diabetics.

Wolff RE, Wolff LF and **Michalowicz BS** [46] determined if HbA1c is elevated in non-diabetic patients with periodontitis. HbA1c was assessed using a chair side test in 59 adults without diabetes but with periodontitis and 53 healthy controls. Unadjusted mean HbA1c levels did not differ significantly between diabetic cases and controls. After adjustments for age, gender, BMI, and current smoking, a higher proportion of cases (27.3%) than controls (13.2%) had HbA1c values $\geq$ 6%, although this difference was not statistically significant (P >0.1). They concluded that periodontitis is associated with a slight elevation in glycosylated hemoglobin.

Fernandes JK et al [47] assessed the prevalence of periodontal disease among a sample of Gullah African Americans with diabetes. Diabetes control was assessed by HbA1c and divided into; well controlled; <7%; moderately controlled; 7% to 8.5%; and poorly controlled; >8.5%. Participants were categorized as healthy (no CAL or BOP), early periodontitis (CAL $\geq$1 mm in at least two teeth), moderate periodontitis (three sites with CAL $\geq$ 4 mm and at least two sites with probing depth [PD] $\geq$ 3 mm), or severe periodontitis (CAL $\geq$ 6 mm in at least two teeth and PD $\geq$ 5 mm in at least one site). They concluded that this population exhibited a higher prevalence of periodontal disease as compared to African Americans. However, diabetes control was not associated with periodontal disease in this population.

Awartani FA [48] investigated the association between glycemic control of type 2 diabetes mellitus (type 2 DM) and severity of periodontal disease (PD) in 126 Saudi diabetic females. 74 patients formed Group I (better control with HbA1c <9%) while 52 formed Group II (poor control with HbA1c >9%). Plaque index, bleeding index, presence of calculus, PPD, and CAL were recorded. The results revealed a significantly higher

percentage of calculus, PD $\geq$ 4 mm and loss of attachment level (3-4 mm) in the poorly controlled diabetic patients, as compared to the better-controlled group.

Chen L et al [49] assessed the relationship of periodontal parameters with metabolic levels and systemic inflammatory markers in 140 patients with type 2 diabetes and periodontitis. Upon an analysis of covariance, subjects with an increased mean PD had significantly higher levels of HbA1c. After controlling for other factors, positive correlations were found between mean PD and HbA1c. After adjustment for possible confounders, the mean PD emerged as a significant predictor variable for elevated level of HbA1c.

MATERIALS AND METHODS

The clearance from Institutional Ethical committee (Annexure 1) was obtained prior to the study and the ethical principles as enumerated in the Helsinki declaration[50] were meticulously followed throughout the course of the study.

I. Sample

Hundred successive patients, 60 with Type 2 diabetics (of more than 3 years duration) from the Diabetology OPD, Government General Hospital, Chennai and 40 non-diabetic patients from the OPD of the Dept. of Periodontology, Tamil Nadu Government Dental College and Hospital, Chennai, were included in this study. All the patients met the following criteria.

Inclusion criteria

1. Generalized chronic periodontitis (clinically defined as CAL in more than 30% of the sites examined)[4] (Figure 1)
2. Age : 30-60 years
3. Voluntary participation and willingness to sign the informed consent

Exclusion criteria

1. Patients with anemia / hematological disorder [6]
2. Patients with Protein Energy Malnutrition
3. Patients with other known systemic disorders
4. Patients requiring antibiotic premedication
5. Patients under steroids
6. Patients with a history of periodontal treatment in the past 6 months
7. Presence of any acute infection

8. Pregnancy

II. Clinical parameters

After a thorough medical history and a signed Informed Consent from the patient (Annexure 2 and 3) the following clinical parameters were evaluated.

1. Plaque Index (Pl I, Silness and Loe, 1964) [51]

All teeth were examined at 4 sites each (disto-facial, facial, mesio-facial, lingual/palatal) and were scored as follows

Score 0 – No plaque

Score 1 – Plaque not visible to the naked eye, detected by explorer

Score 2 – Thin to moderate accumulation of soft deposits within the gingival pocket or on tooth, visible to the naked eye

Score 3 – Abundance of soft matter within gingival pocket or on tooth surface and margin, interdental area stuffed with soft debris

Calculation: Plaque index for a tooth = Total score from 4 areas/ 4

Pl I = Total Plaque indices for all teeth / No. of teeth examined

Interpretation: 0 – Excellent oral hygiene

0.1 to 0.9 – Good oral hygiene

1.0 to 1.9 – Fair oral hygiene

2.0 to 3.0 - Poor oral hygiene

2. Bleeding on Probing (BOP)

For every tooth starting from second molar, the probe was inserted gently into the gingival sulcus at six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual). The appearance of the bleeding at each site indicated a positive score. The total number of bleeding sites per tooth was thus recorded for every tooth except the third molar.

3. Probing Pocket Depth (PPD) (Figure 1)

Probing Pocket Depth was measured from the gingival margin to the base of the pocket in millimeters using Williams Periodontal Probe. The probe was walked within the gingival sulcus along the circumference of the tooth. Six measurements were made per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual, and Distolingual).

4. Clinical Attachment Level (CAL)

Clinical Attachment Level was measured from the Cemento – Enamel Junction (CEJ) to the base of the pocket in millimeter using Williams Periodontal Probe (Figure 2). Three measurements were made on the buccal aspect and three on the lingual aspect of each tooth – total of six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual and Distolingual).

4. Gingival Recession

When the gingival margin was located apical to the CEJ, recession was measured at six sites per tooth (Mesiobuccal, Midbuccal, Distobuccal, Mesiolingual, Midlingual and Distolingual).

5. Attachment Loss Surface Area (ALSA), Periodontal Epithelial Surface Area (PESA) and Periodontal Inflamed Surface Area (PISA)[10,11]

These parameters were derived from Clinical attachment level (CAL), recession and bleeding on probing (BOP) measurements. Excel Spreadsheets that are specially designed for this purpose (Annexure 5) were downloaded and utilized.

To calculate the ALSA, the linear probing measurements, from the cemento–enamel junction (CEJ) to the bottom of the pocket (i.e. CAL), around a particular tooth are fed in the respective Excel cells. Based on the formula function already fed on the excel sheet, these measurements were transformed into the ALSA for that particular tooth. Summing up the individual ALSA scores for the teeth provided the total ALSA score for the patient.

To calculate the PESA, the Recession Surface Area (RSA) was subtracted from ALSA. Since ALSA= PESA + RSA, it was deducted that ALSA – RSA = PESA. To calculate the PESA there are three arithmetical possibilities, depending on the location of the gingival margin (LGM): (Figure 3)

1. When LGM is below CEJ, RSA > 0 and PPD < CAL. Thus PESA < ALSA. Therefore PESA = ALSA- RSA

2. When LGM is exactly at CEJ, PPD = CAL and RSA = 0.

Therefore PESA = ALSA

3. When LGM is above CEJ, PPD > CAL and hence PESA > ALSA.

To calculate PISA, the inflamed part of the PESA, the following steps were followed in the Excel spreadsheet available for this purpose.

1. When the CAL measurements at six sites per tooth are fed in the Excel spreadsheet, the computer calculates the mean CAL for each particular tooth. This is automatically transformed using the appropriate formula for the translation of linear CAL measurements to the ALSA for that specific tooth

2. When the recession measurements at six sites per tooth are fed in appropriate cells, the computer calculates the mean recession for each particular tooth. This is automatically entered into the appropriate formula for the translation of linear recession measurements to the RSA for that specific tooth

3. The computer generates the PESA of a particular tooth based on the above measurements as PESA= ALSA – RSA.

4. The number of sites around the tooth that was affected by BOP is then entered into the designated cells in the worksheet. The PISA for a particular tooth is automatically generated by multiplying PESA by the number of sites with BOP. The sum of all individual PISAs around individual teeth is calculated, amounting to the total PISA within a patient's mouth.

III. Lab investigations

1. Collection of blood sample

Prior to sample collection, skin preparation was done. Five ml of blood sample (Figure 4a, 4b) was drawn from each patient using disposable hypodermic syringe with 23 gauge needle. Blood was collected by venipuncture from antecubital fossa and transported using standardized and aseptic technique (Figure 5). The blood samples were

analyzed for Glycated Hemoglobin (HbA1c) and Glycated Serum Protein **(**Fructosamine) levels. Both the tests were done in Autoanalyzer. (Figure 6)

2. Plasma HbA1c Assay [52]

HbA1c Assay was performed using Diazyme Direct Enzymatic HbA1c kit (Figure 7) in the Institute of Bio-Chemistry, GGH, Chennai-3. The kit consists of lysis buffer, Reagents 1a, 1b, 2 and calibrator which need to be reconstituted before use.

Reagent Preparation -Diazyme HbA1c reagents R1a and R1b were mixed in a 7:3 ratio and allowed to sit at 2-8ºC for 24 hours prior to use. It was mixed gently by inversion, according to the instructions given by the Diazyme laboratories.

Specimen Collection and Handling - The venous whole blood samples were collected with EDTA as anticoagulant. As per the instructions, these samples could be stored by refrigeration and were used within 2 weeks of collection.

Assay Procedure - Whole Blood Bench Top Lysis Procedure

- 250 µL of Lysis reagent was dispensed in an Eppendorf microfuge tube using micropipette (Figure 8).
- Prior to testing, whole blood samples were mixed by gentle inversion at least 5 times to resuspend settled erythrocytes, because accuracy of the assay will be affected if whole blood is not thoroughly mixed prior to testing.
- 20 µL of fully resuspended whole blood sample was added to the lysis buffer (Figure 9). This was mixed gently with a suitable pipette without creating foam and incubated at room temperature (25°C) for 10 min to completely lyse the red blood cells. Complete lysis is observed when the mixture becomes a clear dark red solution without

any particulate matter (Figure 10). The lysate, thus prepared was ready for use in the Direct EnzymaticHbA1c assay and is stable up to 4 hours at room temperature.

- The lysate (20µL of whole blood + 250 µL of the lysis buffer) was then transferred to the sample cup and along with the Reagents R1ab and R2 were run in the autoanalyser. (Figure 11)
- The calibrators were treated exactly as patient samples and were used as per the instructions on labeling.

Results - The HbA1c concentration was expressed directly as % HbA1c by use of a suitable calibration curve in which the calibrators have values for each level in %HbA1c. The values reported are aligned with the Diabetes Control and Clinical Trials (DCCT) system. After addition of Reagent R1, sample and Reagent R2, the result of %HbA1c were reported within 2 min.

3. Glycated Serum Protein Enzymatic Assay (Serum Fructosamine)[52]

GSP assay was done in autoanalyzer using Diazyme Glycated Serum Protein Enzymatic kit (Figure 12) in the Institute of Bio-Chemistry, GGH, Chennai-3.

Reagent Preparation: The kit is supplied with Reagent 1, Reagent 2 and Calibrator.

R1 - As per the instructions, one vial Reagent 1 was reconstituted with 20 mL of distilled water and mixed gently by inversion and then allowed to stand for 24 hours at 2-8°C before use. The reconstituted R1 remains stable for 4 weeks at 2-8°C.

R2 - One vial Reagent 2 was reconstituted with 5 mL of distilled water, mixed gently by inversion and then allowed to stand at room temperature for a minimum of 10 minutes before use. The reconstituted R2 remains stable for 4 weeks at 2-8°C.

Specimen Collection and Handling – The blood samples were centrifuged immediately after collection and serum was separated from cells (Figure 13, 14) and stored in Eppendorf tubes (Figure 15).

Assay Procedure - 20 µL of serum was dispensed into sample cup and run in Autoanalyzer along with Reagent 1 and Reagent 2.

Results - Glycated Serum Protein results are printed out in µmol/L.

All the data collected from patients were entered in a standardized proforma(Annexure 4)

Statistical Analyses

The data for every patient was entered into Excel worksheet and was counter-checked twice before subjecting for data analysis. The normality of the analyzed data was tested with Kolmogorov-Smirnov test. Independent samples t test was used to evaluate if any significant differences existed between the diabetic and non-diabetic groups based on the Age, Plaque Index and ALSA. Differences between the groups for HbA1c, Fructosamine and PISA were assessed for statistical significance with Mann-Whitney test.

Pearson correlation (Bivariate) was used to analyze the strength of association between the investigated variables.[53,54] The correlation coefficient (r) was interpreted as follows.

0.0 - 0.1 - Trivial,

0.1 - 0.3 - Low

0.3 - 0.5 - Moderate

0.5 - 0.7 - High

0.7 - 0.9 - Very high

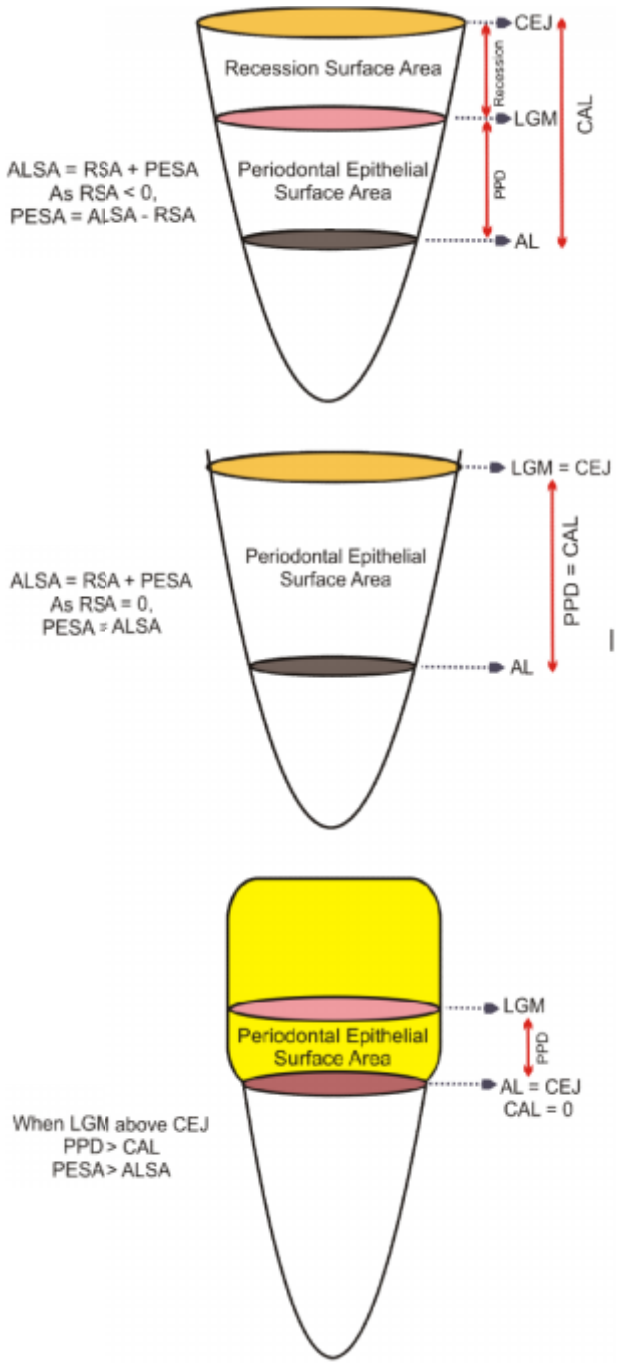
0.9 - 1 - Nearly perfect

Multiple linear regression analysis with backward elimination method was performed to analyze the predictability of ALSA and PISA from the plasma HbA1c or the serum Fructosamine values and other variables evaluated in the study. ALSA or PISA were entered as dependent variables and HbA1c or Fructosamine values, gender, age, duration of diabetes and Plaque Index as independent variables. The significance of the contribution of the variables to the model was estimated and compared with the entry criterion of 0.05 and removal criterion of 0.1 for the probability of F. When a potential predictor met the removal criterion, it was removed from the regression model. The model was then assessed for the remaining predictor variables and the process was continued until no further predictors met the removal criterion. This resulted in the model with minimum number of significant predictor variables.

The alpha level for all the analyses was set at 0.05 and all the statistical procedures were performed in PASW statistics v.18.0.0 (SPSS Inc., 233 South Wacker Drive, 11th Floor Chicago, IL 60606-6412. www.spss.com).

Figure 1: Generalized Chronic Periodontitis

Figure 2: Armamentarium for periodontal examination



AL- Attachment Level; ALSA- Attachment Loss Surface Area; CAL- Clinical Attachment Level;
CEJ- Cemento Enamel Junction; LGM- Location of Gingival Margin;
PESA- Periodontal Epithelial Surface Area; PISA- Periodontal Inflamed Surface Area;
PPD- Probing Pocket Depth; RSA- Recession Surface Area;

Figure 3: Calculation of PESA

Figure 4a: Collection of blood

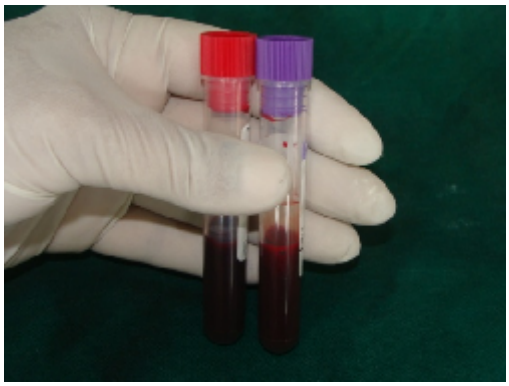


Figure 4b: Collected blood sample

Figure 5: Sample transportation kit

Figure 6: Auto analyzer

Figure 7: HbA1c Kit

Figure 8: Micropipette 5-50µl

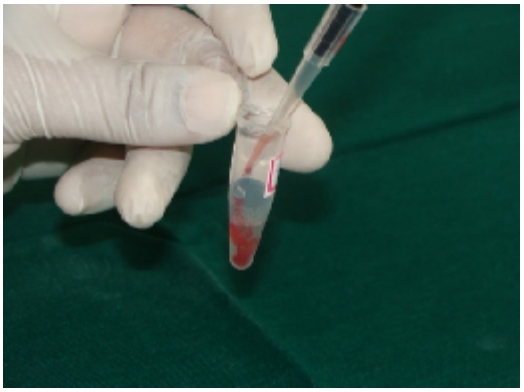


Figure 9: 20 µl of whole blood in lysis buffer

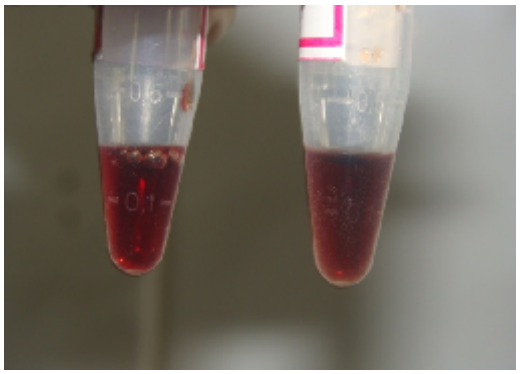


Figure10: The lysed blood

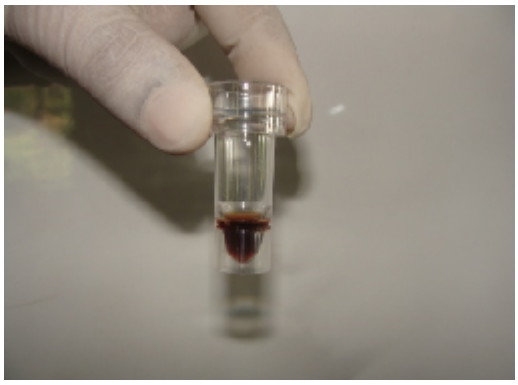


Figure 11: Lysate transferred to sample cup

Figure 12: Fructosamine Kit

Figure 13: Laboratory Centrifuge

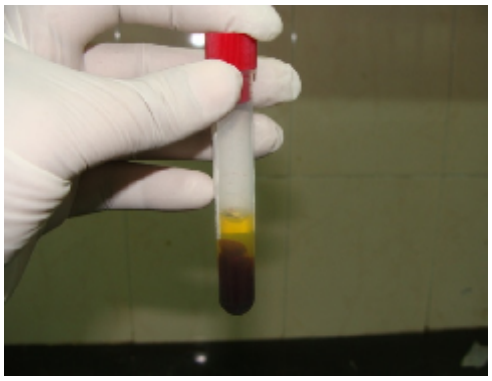


Figure 14 : Centrifuged serum

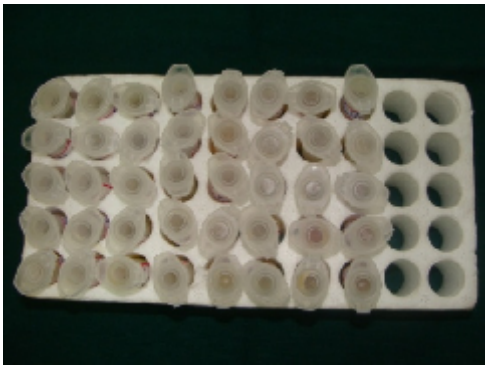


Figure 15: Serum in Eppendorf microfuge tube

RESULTS

The summary statistics for the variables in both the diabetic and non-diabetic group are presented in Table 1. Unpaired t test revealed no statistically significant difference between these groups for age, Plaque index or ALSA. Similarly the inter-group differences in HbA1c, Fructosamine and PISA also failed to reach statistical significance. As expected, the levels of HbA1c and Fructosamine were significantly higher in the diabetic group when compared to non-diabetic group (9.08 ± 1.6 vs. 5.68 ± 0.95 and 3.73 ± 0.97 vs. 2.33 ± 0.52 respectively, $p<0.0001$). Hence it could be concluded that both the groups were well matched except for the diabetic status. A mean HbA1c level of 9.08 ± 1.6 suggested that the glycemic control in the diabetic group was poor.

The results of Pearson correlation test for the diabetic group (Table 2) revealed a very high positive correlation between HbA1c and Fructosamine (0.862, p=0.000). While ALSA correlated highly with both the glycemic markers, the correlations between PISA with HbA1c and Fructosamine were high and moderate respectively. In the non-diabetic group, no statistically significant correlation was evident between any of the glycemic markers and ALSA or PISA (Table 3). However the Plasma HbA1c and Serum fructosamine values exhibited a moderate correlation (r=0.436, p=0.005)

The multiple regression analysis (backward method) revealed that the most significant variables for predicting ALSA in the diabetic group were the glycemic control as indicated by HbA1c or fructosamine, plaque index and age (Tables 4 and 5, Figure 16). The final model for PISA in diabetic group showed HbA1c or fructosamine level and Plaque index to be the most significant predictor variables (Tables 6 and 7, Figure 17). In the non-diabetic group, no predictor variables reached a statistically significant level for

ALSA (Tables 8 and 9). For PISA in the non-diabetic group (Tables 10 and 11), Plaque index emerged as the single most useful predictor, albeit explaining only 14.5% of the variance in PISA.

Table 1. Summary statistics for the Diabetic (n=60) and Non-diabetic (n=40) groups

Parameter	Group	Mean	95% CI mean	Med	Range	SE	SD	p
Age (years)	D ND	51.32 49.25	49.93 – 52.71 47.06 – 51.44	52 49	35 – 60 35 - 60	0.694 1.082	5.38 6.84	0.09 (NS)†
Duration (years)	D	8.26	7.31 – 9.21	7	3 - 20	0.475	3.68	
Plaque Index	D ND	1.32 1.21	1.16 – 1.47 1.08 – 1.33	1.23 1.23	0.52 – 2.76 0.56 – 2.13	0.078 0.062	0.61 0.39	0.32 (NS) †
HbA1c	D ND	9.08 5.68	8.66 – 9.49 5.37 – 5.98	9.20 5.40	5-20 – 11.90 4.50 – 8.00	0.207 0.151	1.60 0.95	<0.0001 ***‡
Fructosamine	D ND	3.73 2.33	3.48 – 3.98 2.16 – 2.50	3.77 2.17	1.39 – 5.40 1.75 – 4.59	0.126 0.083	0.979 0.529	<0.0001 ***‡
ALSA	D ND	2071 2083	1941 – 2202 1892 - 2274	1970 1965	1008 – 3568 1189 - 4482	65.27 94.31	505.6 596.5	0.91 (NS) †
PISA	D ND	981 1172	844.7 – 1117 1634 - 1909	918.2 1116	219.2 - 1955 400.2 - 3276	68.08 83.13	527.7 525.8	0.35 (NS) ‡

D- Diabetic, ND – Non-diabetic, CI – Confidence Interval, Med – Median, SE – Standard Error, SD – Standard Deviation, p – Probability value (two-tailed),

NS – Non-significant, ***- P<0.001

† - Unpaired t test, ‡ - Mann-Whitney U test

Table 2. Pearson correlation (Bivariate) for variables in the Diabetic group (n=60)

		Age	Gender	Duration	HbA1c	FA	Pl I	PISA	ALSA
Age	r	1	-.101	.290*	.152	.177	.007	.083	.276*
	p		.443	.024	.247	.176	.956	.526	.033
Gender	r	-.101	1	-.027	-.057	-.021	-.092	-.177	-.116
	p	.443		.838	.664	.874	.483	.175	.376
Duration	r	.290*	-.027	1	-.035	-.004	.084	.065	-.015
	p	.024	.838		.793	.976	.522	.624	.910
HbA1c	r	.152	-.057	-.035	1	.862***	.265*	.545***	.614***
	p	.247	.664	.793		.000	.041	.000	.000
FA	r	.177	-.021	-.004	.862***	1	.167	.445***	.513***
	p	.176	.874	.976	.000		.203	.000	.000
Pl I	r	.007	-.092	.084	.265*	.167	1	.598***	.529***
	p	.956	.483	.522	.041	.203		.000	.000
PISA	r	.083	-.177	.065	.545***	.445***	.598***	1	.679***
	p	.526	.175	.624	.000	.000	.000		.000
ALSA	r	.276*	-.116	-.015	.614***	.513***	.529***	.679***	1
	p	.033	.376	.910	.000	.000	.000	.000	

r - Pearson correlation coefficient, p – Probability value (two tailed)

*- p,0.05, ***- P<0.001

Table 3. Pearson correlation (Bivariate) for variables in the Non-Diabetic group (n=40)

		Age	Gender	Hba1c	FA	Pl I	PISA	ALSA
Age	r	1	-.118	.247	.080	.113	-.198	-.171
	p		.469	.125	.625	.489	.221	.292
Gender	r	-.118	1	.099	.060	.062	-.142	-.130
	p	.469		.542	.712	.703	.384	.423
HbA1c	r	.247	.099	1	.436**	.371*	.170	.053
	p	.125	.542		.005	.018	.293	.746
FA	r	.080	.060	.436**	1	.554***	.292	.103
	p	.625	.712	.005		.000	.068	.527
Pl I	r	.113	.062	.371*	.554***	1	.381*	.169
	p	.489	.703	.018	.000		.015	.296
PISA	r	-.198	-.142	.170	.292	.381*	1	.830***
	p	.221	.384	.293	.068	.015		.000
ALSA	r	-.171	-.130	.053	.103	.169	.830***	1
	p	.292	.423	.746	.527	.296	.000	

r - Pearson correlation coefficient, p – Probability value (two tailed)

*- p,0.05, **-p<0.01 ***- P<0.001

Table 4. Multiple regression (backward) for ALSA with HbA1c in diabetic group

Dependent variable – ALSA
Model 1 – Predictors: (Constant), Duration, Gender, HbA1c, Pl I, Age
Model 2 – Predictors: (Constant), Duration, HbA1c, Pl I, Age
Model 3 - Predictors: (Constant), HbA1c, Pl I, Age

Table 4A. Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.756	.571		.531	346.072	.000
2	.755	.570	-0.001	.539	343.3007	.000
3	.749	.561	-0.009	.538	343.827	.000

Table 4B – Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	SE	Beta		
1	Constant	-673.171	488.001		-1.379	.173
	HbA1c	146.236	29.593	.465	4.942	.000
	Pl I	338.949	77.195	.410	4.391	.000
	Age	21.506	8.936	.229	2.407	.020
	Gender	-32.474	92.778	-.031	-.350	.728
	Duration	-13.814	12.899	-.101	-1.071	.289
2	Constant	-712.742	470.923		-1.514	.136
	HbA1c	146.419	29.352	.466	4.988	.000
	Pl I	341.226	76.305	.413	4.472	.000
	Age	21.802	8.825	.232	2.470	.017
	Duration	-13.852	12.796	-.101	-1.083	.284
3	Constant	-697.679	471.439		-1.480	.145
	HbA1c	149.928	29.217	.477	5.132	.000
	Pl I	331.930	75.936	.402	4.371	.000
	Age	18.895	8.420	.201	2.244	.029

Table 5. Multiple regression (backward) for ALSA with Fructosamine in diabetic group

Dependent variable – ALSA
Model 1 – Predictors: (Constant), Duration, FA, Gender, Pl I, Age
Model 2 – Predictors: (Constant), Duration, FA, Pl I, Age
Model 3 - Predictors: (Constant), FA, Pl I, Age

Table 5A – Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.722	.521		.477	365.63510	.000
2	.721	.519	-0.002	.484	363.02943	.000
3	.711	.506	-0.014	.479	364.82994	.000

Table 5B – Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	Constant	-159.856	489.692		-.326	.745
	FA	202.503	50.232	.392	4.031	.000
	Pl I	387.299	79.668	.469	4.861	.000
	Age	22.034	9.467	.235	2.327	.024
	Gender	-45.857	98.015	-.044	-.468	.642
	Duration	-16.754	13.583	-.122	-1.233	.223
2	Constant	-213.719	472.574		-.452	.653
	FA	202.189	49.869	.392	4.054	.000
	Pl I	390.785	78.753	.473	4.962	.000
	Age	22.477	9.353	.239	2.403	.020
	Duration	-16.827	13.486	-.123	-1.248	.217
3	Constant	-178.494	474.070		-.377	.708
	FA	206.826	49.977	.401	4.138	.000
	Pl I	381.224	78.768	.461	4.840	.000
	Age	18.989	8.969	.202	2.117	.039

Table 6. Multiple regression (backward) for PISA with HbA1c in diabetic group

Dependent variable – PISA
Model 1 - Predictors: (Constant), Duration, Gender, HbA1c, Pl I, Age
Model 2 - Predictors: (Constant), Duration, Gender, HbA1c, Pl I
Model 3 - Predictors: (Constant), Gender, HbA1c, Pl I
Model 4 - Predictors: (Constant), HbA1c, Pl I

Table 6A – Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.729	.531		.488	377.428	.000
2	.729	.531	0.000	.497	373.990	.000
3	.728	.530	-0.001	.505	371.138	.000
4	.720	.518	-0.012	.501	372.505	.000

Table 6B – Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	SE	Beta		
1	Constant	-740.814	532.217		-1.392	.170
	HbA1c	135.879	32.275	.414	4.210	.000
	Pl I	409.528	84.189	.475	4.864	.000
	Age	-.469	9.746	-.005	-.048	.962
	Gender	-117.673	101.184	-.109	-1.163	.250
	Duration	5.337	14.068	.037	.379	.706
2	Constant	-761.362	315.077		-2.416	.019
	HbA1c	135.603	31.469	.414	4.309	.000
	Pl I	409.830	83.191	.475	4.926	.000
	Gender	-117.212	99.812	-.109	-1.174	.245
	Duration	5.131	13.282	.036	.386	.701
3	Constant	-715.856	290.002		-2.468	.017
	HbA1c	134.874	31.173	.411	4.327	.000
	Pl I	412.882	82.183	.479	5.024	.000
	Gender	-118.034	99.028	-.110	-1.192	.238
4	Constant	-810.498	279.947		-2.895	.005
	HbA1c	136.140	31.269	.415	4.354	.000
	Pl I	420.736	82.221	.488	5.117	.000

Table 7. Multiple regression (backward) for PISA with Fructosamine in diabetic group

Dependent variable – PISA
Model 1 - Predictors: (Constant), Duration, FA, Gender, Pl I, Age
Model 2 - Predictors: (Constant), Duration, FA, Gender, Pl I
Model 3 - Predictors: (Constant), FA, Gender, Pl I
Model 4 - Predictors: (Constant), FA, Pl I

Table 7A - Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.704	.495		.448	391.700	.000
2	.704	.495	0.000	.458	388.123	.000
3	.703	.495	0.000	.468	384.769	.000
4	.693	.480	-0.015	.462	386.846	.000

Table 7B – Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	Constant	-268.513	524.601		-.512	.611
	FA	190.968	53.813	.355	3.549	.001
	Pl I	453.677	85.347	.526	5.316	.000
	Age	-.081	10.142	-.001	-.008	.994
	Gender	-130.181	105.003	-.121	-1.240	.220
	Duration	2.662	14.552	.019	.183	.856
2	Constant	-272.170	251.491		-1.082	.284
	FA	190.886	52.326	.355	3.648	.001
	Pl I	453.717	84.416	.526	5.375	.000
	Gender	-130.097	103.521	-.121	-1.257	.214
	Duration	2.627	13.761	.018	.191	.849
3	Constant	-251.317	224.578		-1.119	.268
	FA	190.702	51.865	.354	3.677	.001
	Pl I	455.072	83.391	.528	5.457	.000
	Gender	-130.480	102.607	-.121	-1.272	.209
4	Constant	-345.793	213.077		-1.623	.110
	FA	191.077	52.144	.355	3.664	.001
	Pl I	464.629	83.500	.539	5.564	.000

Table 8. Multiple regression (backward) for ALSA with HbA1c in Non-diabetic group

Dependent Variable: ALSA
Model 1 - Predictors: (Constant), Gender, Pl I, Age, HbA1c
Model 2 - Predictors: (Constant), Gender, Pl I, Age
Model 3 - Predictors: (Constant), Pl I, Age
Model 4 - Predictors: (Constant), Age
Model 5 - Predictor: (constant)

Table 8A. Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.309	.096		-.008	598.819	.461
2	.305	.093	-0.003	.017	591.317	.314
3	.255	.065	-0.028	.015	592.117	.288
4	.171	.029	-0.036	.004	595.418	.292
5	.000	.000	-0.029	.000	596.491	

Table 8B. Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	SE	Beta		
1	Constant	2634.038	823.255		3.200	.003
	HbA1c	35.809	111.205	.058	.322	.749
	Pl I	276.938	260.417	.184	1.063	.295
	Age	-19.711	14.621	-.226	-1.348	.186
	Gender	-207.499	194.710	-.174	-1.066	.294
2	Constant	2743.147	740.892		3.702	.001
	Pl I	306.332	240.838	.204	1.272	.212
	Age	-18.603	14.033	-.213	-1.326	.193
	Gender	-200.347	191.016	-.168	-1.049	.301
3	Constant	2560.078	721.011		3.551	.001
	Pl I	287.032	240.459	.191	1.194	.240
	Age	-16.751	13.940	-.192	-1.202	.237
4	Constant	2815.405	692.392		4.066	.000
	Age	-14.876	13.928	-.171	-1.068	.292
5	Constant	2082.783	94.314		22.084	.000

Table 9. Multiple regression (backward) for ALSA with Fructosamine in Non-diabetic group

Dependent Variable: ALSA
Model 1 - Predictors: (Constant), Gender, FA, Age, Pl I
Model 2 - Predictors: (Constant), Gender, Age, Pl I
Model 3 - Predictors: (Constant), Age, Pl I
Model 4 - Predictors: (Constant), Age
Model 5 - Predictor: (constant)

Table 9A. Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	AVOVA p value
1	.305	.093		-.010	599.563	.474
2	.305	.093	0.000	.017	591.318	.314
3	.255	.065	-0.028	.015	592.117	.288
4	.171	.029	-0.036	.004	595.418	.292
5	.000	.000	-0.029	.000	596.491	

Table 9B. Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	SE	Beta		
1	Constant	2705.342	806.224		3.356	.002
	FA	28.123	217.721	.025	.129	.898
	Pl I	285.696	291.816	.190	.979	.334
	Age	-18.649	14.233	-.214	-1.310	.199
	Gender	-201.197	193.791	-.169	-1.038	.306
2	Constant	2743.147	740.892		3.702	.001
	Pl I	306.332	240.838	.204	1.272	.212
	Age	-18.603	14.033	-.213	-1.326	.193
	Gender	-200.347	191.016	-.168	-1.049	.301
3	Constant	2560.078	721.011		3.551	.001
	Pl I	287.032	240.459	.191	1.194	.240
	Age	-16.751	13.940	-.192	-1.202	.237
4	Constant	2815.405	692.392		4.066	.000
	Age	-14.876	13.928	-.171	-1.068	.292
5	Constant	2082.783	94.314		22.084	.000

Table 10. Multiple regression (backward) for PISA with HbA1c in Non-diabetic group

Dependent Variable: PISA
Model 1 - Predictors: (Constant), Gender, Pl I, Age, HbA1c
Model 2 - Predictors: (Constant), Gender, Pl I, Age
Model 3 - Predictors: (Constant), Pl I, Age
Model 4 - Predictors: (Constant), Pl I

Table 10A. Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.505	.255		.170	478.879	.031
2	.493	.243	-0.012	.180	476.040	.017
3	.452	.204	-0.039	.161	481.530	.015
4	.381	.145	-0.059	.123	492.400	.015

Table 10B. Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	SE	Beta		
1	Constant	1346.112	658.361		2.045	.048
	HbA1c	67.404	88.931	.123	.758	.454
	Pl I	506.461	208.257	.382	2.432	.020
	Age	-22.772	11.693	-.296	-1.948	.060
	Gender	-223.095	155.711	-.212	-1.433	.161
2	Constant	1551.491	596.455		2.601	.013
	Pl I	561.791	193.887	.424	2.898	.006
	Age	-20.686	11.297	-.269	-1.831	.075
	Gender	-209.633	153.778	-.200	-1.363	.181
3	Constant	1359.936	586.352		2.319	.026
	Pl I	541.597	195.549	.409	2.770	.009
	Age	-18.748	11.336	-.244	-1.654	.107
4	Constant	480.754	252.943		1.901	.065
	Pl I	505.157	198.690	.381	2.542	.015

Table 11. Multiple regression (backward) for PISA with Fructosamine in Non-diabetic group

Dependent Variable: PISA
Model 1 - Predictors: (Constant), Gender, FA, Age, Pl I
Model 2 - Predictors: (Constant), Gender, Age, Pl I
Model 3 - Predictors: (Constant), Age, Pl I
Model 4 - Predictors: (Constant), Pl I

Table 11A. Model Summary

Model	R	R Square	R square change	Adjusted R Square	SE of Estimate	ANOVA p value
1	.505	.255		.170	478.997	.032
2	.493	.243	-0.012	.180	476.040	.017
3	.452	.204	-0.039	.161	481.530	.015
4	.381	.145	-0.059	.123	492.400	.015

Table 11B. Coefficients

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	Constant	1376.998	644.101		2.138	.040
	FA	129.803	173.940	.131	.746	.460
	Pl I	466.541	233.135	.352	2.001	.053
	Age	-20.898	11.371	-.272	-1.838	.075
	Gender	-213.554	154.822	-.203	-1.379	.177
2	Constant	1551.491	596.455		2.601	.013
	Pl I	561.791	193.887	.424	2.898	.006
	Age	-20.686	11.297	-.269	-1.831	.075
	Gender	-209.633	153.778	-.200	-1.363	.181
3	Constant	1359.936	586.352		2.319	.026
	Pl I	541.597	195.549	.409	2.770	.009
	Age	-18.748	11.336	-.244	-1.654	.107
4	Constant	480.754	252.943		1.901	.065
	Pl I	505.157	198.690	.381	2.542	.015

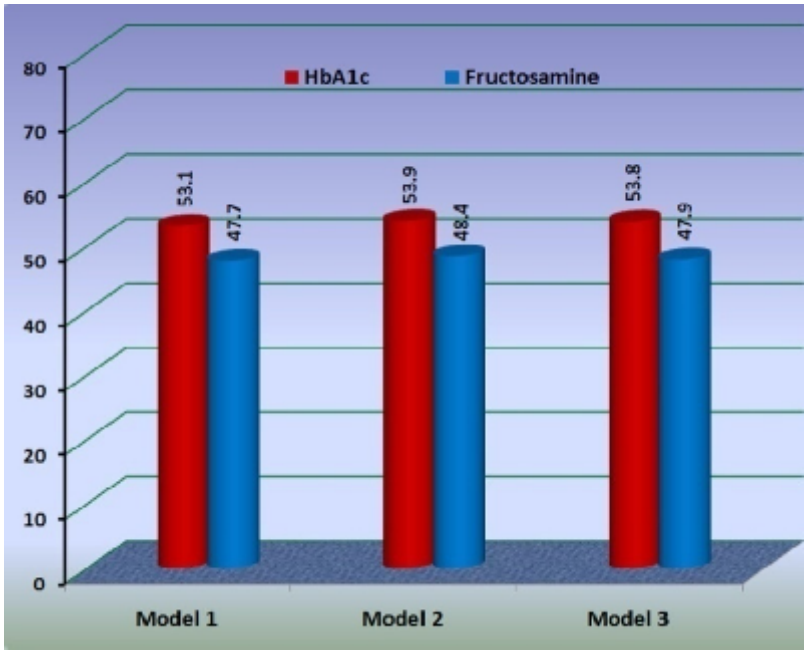


Figure 16: Percentage of variation in ALSA accounted by the Regression models for the diabetic group.

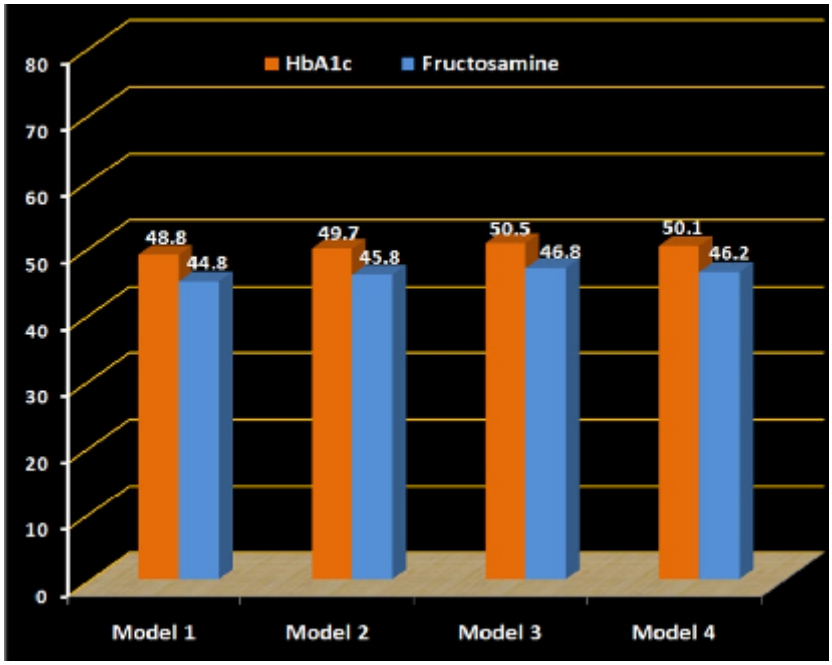


Figure 17: Percentage of variation in PISA accounted by the Regression models for the diabetic group.

DISCUSSION

Diabetic patients are more susceptible to gingivitis and periodontitis than healthy subjects.[55,56,57,58] GCF glucose levels, periodontal vasculature, host response and collagen metabolism are among the proposed mechanisms by which diabetes may affect the periodontium [59]. In hyperglycemic state, several proteins and matrix molecules undergo a non-enzymatic glycosylation resulting in Advanced Glycation End Products (AGEs). These AGEs play a central role in the classic complications of diabetes[60] including the progression of the periodontal disease.[61] Hence it is critical to evaluate the level of glycemic control in a diabetic patient, before initiating periodontal treatment.

From a periodontal point of view, random glucose and fasting blood glucose are of limited value since they indicate the glucose concentration at the point of time. The most common of the available tests for monitoring the long and short term control of glucose are the estimation of Glycated Hemoglobin or plasma HbA1c and Glycated Albumin or serum fructosamine respectively.[34] These are generated by a non-enzymatic attachment of free carbonyl group of glucose through a Schiff base- Amadori rearrangement at multiple sites on polypeptide chains of hemoglobin and albumin respectively.[8,62] While HbA1c assay indicates the plasma glucose over 6-8 weeks (for the average half-life of RBC is 60 days)[28], Fructosamine reflects the glycemic conditions during the preceding 1-3 weeks (half-life of albumin being 14-20 days).

Several studies have revealed correlation between plasma HbA1c and periodontal disease severity.[43,44,47,48,63] However, there is a scarcity of literature investigating the association of the HbA1c with serum fructosamine status. Further, many studies comparing HbA1c and Fructosamine levels with periodontal diseases severity, have expressed periodontitis as a non-continuous variable such as mild, moderate, severe

etc.[10,11] or in terms of probing pocket depth and clinical attachment level. By definition, these classifications do not quantify the amount of diseased or inflamed periodontal tissue.[10] Apparently only two studies are available that has utilized Attachment Loss Surface Area (ALSA) or Periodontal Inflamed Surface Area (PISA) , the recently introduced parameters that express the periodontal disease severity in truly quantitative manner.[10,11] The acute scarcity of literature for the comparative association of HbA1c versus Fructosamine levels with periodontal disease severity, especially when the latter is expressed in terms of ALSA and PISA, substantiates the need for and the appropriateness of the current study.

Correlation between Glycemic control, ALSA and PISA

The results of bivariate correlation test (Pearson) in the present study showed that Plasma HbA1c had a very high correlation with Serum Fructosamine levels in the diabetic group (r = 0.862, p=0.000, Table 2). Similar results have been reported by Baker at al[64] (r=0-82; p<0.001), Ludvigsen et al[24] (r = 0.890), Narbonne et al[65](r= 0.88, p,0.01) and Tahara[66] (r = 0.747, p < 0.001) among diabetic patients. In contrast Ko et al[67] found only a low level of correlation between them (r = 0.335, p<0.001) when adjusted for age. In general, there appears to be a satisfactory level of correlation between HbA1c and Fructosamine in diabetic patients thereby allowing the estimation of the former from Fructosamine.

When the correlation between glycemic control and ALSA was assessed in the diabetic patients in this study, plasma HbA1c had a marginally better correlation than Fructosamine, though both fell under 'high' category (r =0.614, p = 0.000 and r = 0.513, p = 0.000 respectively). Lack of any similar study in the literature has left no scope for

the direct comparison of these results. Some of the earlier researches on the relation between periodontal disease severity and glycemic control have found no association between them. In an investigation on 26 type I diabetic patients , Pinson et al[35] found no significant association between the level of control of diabetes (Glycated Hb) and probing depths, clinical attachment levels or recession. Similar results have been reported by Alpagot et al [41] and Bridges et al.[68]

However the majority of later studies have identified a positive correlation between the two. Negishi J et al [69] detected the high value of HbA1c to be significant factor related to advanced periodontitis in diabetic patients. Even among 12- to 18-year-old diabetic children, glycemic control as indicated by HbA1c remained a highly significant correlate of periodontitis. A positive correlation, has been reported between mean PD and HbA1c in diabetic cases by Chen et al [49] ($r = 0.2272$; $p = 0.009$) and Lim et al [70] ($r=0.26$, $p<0.05$). In a study on 126 female type 2 diabetics, Awartani F [48] found a significant association between the loss of attachment level (3-4 mm) and poor control of diabetes. Slightly higher correlation values have been reported by Firatli et al [37] for Serum fructosamine with PPD and CAL (0.23 and 0.32) than for Plasma HbA1c (0.13 and 0.27). However no information regarding the statistical significance of this data has been presented. Overall, there appears to be a positive correlation between plasma HbA1c and PPD or CAL in diabetic patients. ALSA is a variable that quantifies the cumulative periodontal destruction in an individual and is a derivation from either PPD or CAL. Hence it is only logical that it shows a correlation with HbA1c or Fructosamine similar to PPD and CAL.

The evaluation of correlation between PISA and glycemic control in diabetic cases of the present study showed results analogous to ALSA, with HbA1c exhibiting a better correlation. In terms of strength of the correlation, that of HbA1c with PISA was high ($r = 0.545$, $p = 0.000$) and that of Fructosamine was 'moderate' ($r = 0.445$, $p = 0.000$). PISA is a continuous variable that quantifies the extent of active periodontal disease in an individual and hence systemic burden resulting from it. It is a product of periodontal pocket area (a derivation from PPD) and the number of sites with bleeding on probing. PPD has been shown to correlate positively with HbA1c in several studies. Similarly bleeding on probing has also been found to be significantly associated with glycemic control. The vascular changes in diabetes mellitus result in increased gingival bleeding with the thickening of the basement membrane of the small vessels in the gingival tissues being one of them.[71,72] Lu et al[63] found that the mean Gingival Index values for Type 2 diabetics with HbA1c $\geq$ 10% were more than those with HbA1c < 10 %. Serum fructosamine showed positive correlation with gingival index in both insulin-dependent diabetic children and adolescents ($r=0.68$)[73] and NIDDM patients ($r = 0.684$).[34] Gingival collagenase activity has been shown to reach a maximum in 15-22 days after the development of gingivitis in diabetes. The span of fructosamine turnover approximately coinciding with this activation period of gingival collagenase has been put forward as the possible explanation of high correlation between serum fructosamine and gingival bleeding.[34] Given that PISA represents the pocket area with bleeding on probing and that glycemic control shows good correlation with both PPD and BOP, the correlation between HbA1c and fructosamine with PISA appears plausible.

The results of non-diabetic controls showed a moderate correlation between HbA1c and fructosamine levels (r= 0.436, p=0.005, Table 3), but lesser than that observed for the diabetic group. This is in agreement with Misciagna et al [74], who have reported a similar correlation between fructosamine and HbA1c (r= 0.41) in non-diabetics. However, Narbonne H et al [65] found a good correlation (r = 0.88) only in diabetic patients and not in control subjects (r = 0.01). As for the relation between glycemic control and periodontal disease severity in the current sample, HbA1c or serum Fructosamine levels showed a positive, but trivial, correlation with ALSA (r=0.053, p=0.746 and r= 0.103,p=0.527 respectively). Both exhibited a mild correlation (r=0.170, p=0.293 and r= 0.292,p=0.068 respectively) with PISA. Similar are the findings of Hayashida et al [45] and Wolf et al [46] who found a significant relationship between periodontal status and Plasma HbA1c levels in non-diabetic individuals.

Though HbA1c is considered as the "gold standard" for assessing glycemic control, a number of hemoglobinopathies cause false results in HbA1c determinations, thus presenting a unique challenge to clinical practitioners. Several conditions may affect HbA1c determination include decreased lifespan of red blood cells secondary to massive bleeding, hemolytic anemia, chronic disease or pregnancy induced anemia. In addition, other causes affecting HbA1c determination are uremia, opiate addiction, chronic alcohol abuse, high dose aspirin, hyperbilirubinemia, vitamin C excess, vitamin E excess, iron deficiency anemia or hypertriglyceridemia.[6] In such cases, serum fructosamine provides an alternative glycemic marker. Fructosamine responds much sooner to changes in glycemic control level, enabling us to evaluate the treatment regimen sooner, since it

represents the time averaged plasma glucose level over 2–4 weeks [66]. The other added advantages include low cost and simple laboratory procedure [9].

Considering, a satisfactory level of correlation between these glycemic markers and either of them with periodontal disease status, Fructosamine appears a justifiable alternative for HbA1c in generalized chronic periodontitis patients with Type 2 diabetes mellitus.

Prediction models for ALSA and PISA

The current study explored the predictability of ALSA and PISA from the glycemic control (HbA1c or Fructosamine), duration of diabetes (not applicable in the non-diabetics), Age, gender and Plaque index of the individual using multiple linear regression analysis. ALSA or PISA was entered as the independent variable and Age, Gender, HbA1c or Fructosamine, duration of diabetes and Plaque index were introduced as the predictor variables. A backward method was used for the selection of the predictor variables in which all the predictor variables are entered into the model. The weakest predictor variable is removed from the model and the regression re-calculated. If this does not significantly weaken the model, the predictor variable is deleted. This is repeated until the final model which contains only the useful predictors is produced.[75]

In the present study, the regression analysis for ALSA using HbA1c in diabetic group resulted in a final model containing Plaque index, HbA1c and Age as the significant predictor variables accounting for 53.8 % of the variance in ALSA (Table 4). Gender and duration of diabetes had contributed only 1% (r^2 change of 0.010) to the variance in ALSA and hence were eliminated from the final model. HbA1c appeared to

be the most influential variable followed closely by Plaque Index and then by Age in decreasing order. A similar model resulted when the regression analysis was run with fructosamine in place of HbA1c with Plaque Index, Fructosamine and Age being significant predictors in the decreasing order of relative influence on ALSA (Table 5). However this model accounted for a slightly lesser amount of variance in ALSA (47.9%) than HbA1c final model. Of three variables, Plaque index had the highest impact on ALSA followed closely by serum fructosamine level.

In general, the glycemic control, the local environment (as represented by plaque index in this study) and age appeared to be the important variables on predicting the ALSA, a measure of the cumulative periodontal destruction in an individual. This supports Novak and Novak's enlisting of tobacco smoking, diabetes, pathogenic bacteria and microbial tooth deposits as the main risk factors for periodontal disease.[4] Genetic factors, age, gender, socioeconomic status and stress have been put forward as the background characteristics that affect the periodontal disease status.[4] Smoking has clearly been identified as the best predictor of attachment loss in diabetic patients.[43,44] While few reports have failed to observe a significant association between glycemic control and periodontal disease,[27,31,35,76,77,78,79] and many have reported a significant relationship between the two.[32, 47,70,80-82] The role of plaque and calculus in having a pathogenic effect on the progression of periodontal disease has also been well documented.[42,83-86] Increased prevalence and severity of periodontal disease has been reported in old age, with the attachment loss and bone loss in these individuals seen as a cumulative effect of the result of prolonged exposure to other risk factors over a person's life.[87,88] The final model of regression analysis for ALSA in the study corroborates the findings on the

causative or contributory role of glycemic control in diabetes along with plaque and age in the cumulative effect of periodontal disease in an individual.

The negligible effect of duration of diabetes on periodontal disease severity are in agreement with Bridges et al [68], Rylander et al [76], Ervasti et al [80], who did not find relationship between the two. On the contrary, Firatli E [37] and Lu HK [63] have shown positive correlation between duration of diabetes and CAL. Similarly, the negligible contributory influence of gender on the periodontal disease severity is also in contrast to certain reports of increased loss of attachment in males.[4,84] As smoking was as an exclusion criteria for the samples in the study, its contributory effect could not be evaluated. This factor could probably be a major contributor towards the nearly 50% of the variance in ALSA that remains unexplained in the final prediction models of the study.

In our study, the HbA1c and Fructosamine based regression models for PISA showed these measures along with Plaque index accounted 50.1% and 46.2% of the variance in PISA respectively (Table 6, Table 7). Akin to the condition for ALSA, gender and duration of diabetes had either a very meager or no effect on the variance of PISA. However, unlike for ALSA, age did not show any causal influence towards the variance in PISA. Age related recession has been the primary modus through which age contributes to periodontal disease severity measured in terms of CAL. Recession is not a consideration in the calculation of PISA and hence the absence of age as in the final predictive model for PISA is not surprising. PISA, by definition, is the pocket surface that bleeds on probing indicating the sites with active disease. The role of microbial plaque in causing gingivitis is well established, with bleeding on probing being the

cardinal sign. The vascular changes in diabetes mellitus resulting in increased gingival bleeding have also been well documented.[72,89] Many studies have reported a higher gingival index in diabetics as compared to controls.[34,63,73] Hence, the appearance of Plaque index and HbA1c / fructosamine level as the most important variables for predicting PISA appears reasonable. Nesse et al [11] assessed a dose–response relationship between PISA and HbA1c levels in forty type 2 diabetics. After controlling for factors that might influence PISA or HbA1c levels, the higher the PISA of type 2 diabetics was, the higher their HbA1c levels were. On a group level, an increase of PISA with 333 mm^2 was associated with a 1.0 percentage point increase of HbA1c, independent of the influence of other factors, thus suggesting a causal relationship between periodontitis and diabetes mellitus.

In the non-diabetic group with generalized chronic periodontitis, the backward multiple regression analysis could not find any statistically significant model for predicting ALSA from HbA1c or Fructosamine (Table 8, Table 9). For the prediction model of PISA both HbA1c and Fructosamine were eliminated as the first variable. Plaque index emerged as the single most important predictor accounting for 12.3% of the variance in PISA with glycemic status, gender and age together contributing to 11% of the variance (Table 10, Table 11). The effect of microbial plaque resulting in gingivitis and thereby gingival bleeding could be the possible explanation for this result.

One main methodological advantage of this study is the use of enzymatic assay method to estimate the levels of plasma HbA1c and serum Fructosamine. Many studies in the periodontal literature, have utilized High Performance Liquid chromatographic method for the estimation of glycosylated hemoglobin.[37, 45, 70, 90] A chair side method for

determination of HbA1c (A1c Now+, Bayer Health Care) has been reported by Wolff et al.[46] Overall, a majority of the laboratory methods require dual channel testing, i.e. separate tests are required for glycated hemoglobin (GHb) and for total hemoglobin (THb). The final %HbA1c value is expressed as a ratio of the specific GHb in relation to the THb found in the whole blood sample. However, the Direct Enzymatic HbA1c Assay used in the present study, is a single channel test and reports % HbA1c values directly, without the need for a separate Total Hb test or a calculation step. It has all the advantages of both the HPLC and immunoassays methods in the areas of accuracy, specificity, applicability to chemistry analyzers and yet is cost effective, simpler and has less interference. Moreover, enzymatic HbA1c assays are neither interfered by chemical or genetically modified hemoglobin variants nor reports false results regardless of the patient's hemoglobin variant types and are therefore highly reliable.

Similar to HbA1c, the serum Fructosamine was also evaluated using Diazyme Enzymatic Fructosamine assay.[26] Unal et al[34] and Firatli et al[73] have used Nitroblue Tetrazolium (NBT) colorimetric procedure to determine Fructosamine levels. With a mean intra assay <2% and mean inter-assay CV of <3%,[52] the enzymatic assay used in this study has definite advantages of improved specificity and reliability over the conventional NBT-based method.

The majority of the studies and most of the indices quantifying the severity of periodontal disease use limited number of sites or teeth in the patients mouth (most often the Ramjford teeth -11,16, 24, 31, 36, 44) [63] thereby intrinsically introducing a possibility of under or overestimation of the condition. Assessing at six sites per tooth and inclusion

of all the teeth (except third molars), as done in the current study is likely to be more accurate than those from few representative teeth.

Limitations and future scope

A few limitations of the study require special consideration, the most significant being a relatively low sample size of 60 type 2 diabetes and 40 non-diabetics. Hence the results of the study cannot be generalized without reservations. In addition, the mean plasma HbA1c and Serum Fructosamine values of the diabetic group in this study indicated a poor glycemic control. Therefore the models derived here may be better representative of the association between glycemic control and periodontal disease severity in poorly controlled diabetes mellitus. The applicability of these models in patients with good glycemic control requires further scrutiny.

The effect of smoking and other variables like socioeconomic status and stress have not been evaluated in the current study. Including them could have resulted in a more comprehensive prediction model for ALSA and PISA.

PISA, as a periodontal parameter, has some inherent disadvantages. PISA quantifies the amount of inflamed periodontal tissue in two dimensions, whereas periodontitis is a three dimensional inflammatory process, i.e., it extends into the connective tissue around the root. However, the same is applicable even for conventional parameters like CAL, BOP, PPD etc. Hence it can be contended that , though not precise, ALSA and PISA quantifies the amount of affected periodontal tissue more accurately than any classification currently used (face validity).

The formulas used to derive ALSA and PISA in the excel spreadsheets devised by Nesse et al[10] have used means for root lengths and root surface areas derived from their population. Thus individual and population variations in these variables have been overlooked when calculating ALSA and PISA. This could be addressed partially by utilizing the mean root surface areas and root lengths in our population.

Due to high variation in the development and eruption, the third molars have not been included in the calculation of ALSA and PISA. Though this would impart greater convenience and consistency in the clinical readings, it could have lead to slight underestimation of ALSA and PISA in whom they are erupted and functional.

SUMMARY & CONCLUSIONS

The association between the two markers of Glycemic control, Plasma HbA1c and Serum Fructosamine levels and periodontal disease severity expressed in terms of Attachment Loss Surface Area (ALSA) and Periodontal Inflamed Surface Area (PISA) were evaluated in 60 type 2 diabetics and 40 non-diabetic patients with generalized chronic periodontitis.

Within the limitations the study, the following conclusions were drawn

1. Plasma HbA1c and Serum Fructosamine exhibited a high correlation in generalized chronic periodontitis patients with in type 2 diabetes mellitus and a moderate level of correlation in periodontitis patients without diabetes.

2. Both these markers exhibited a marginally better correlation with ALSA than with PISA in type 2 diabetics.

3. Albeit in the comparable range, Plasma Hba1c showed slightly higher correlation than Serum Fructosamine, both with ALSA and PISA in type 2 diabetics

4. No significant correlation was observed in non-diabetics, for either of these markers of glycemic control with ALSA or PISA.

5. Glycemic control as indicated by Plasma HbA1c or Serum Fructosamine, Plaque index and Age appeared to be the important predictor variables for ALSA accounting for nearly half of its variance.

6. HbA1c and Fructosamine based regression models for PISA in diabetic patients showed that these measures along with plaque index accounted for 50.1% and 46.2% of the variance in PISA respectively.

7. None of the variables evaluated in the study (HbA1c/ Fructosamine, Plaque index, Age, Gender and Duration of diabetes) were significantly predictive of ALSA in the non-diabetic group. The most significant variable for predicting PISA in this group was Plaque index, although it accounted only to 10 % of variance in PISA.

Considering the high association between Plasma HbA1c and Serum Fructosamine and a comparable level of association of ALSA and PISA with either of them, it seems justified to use Fructosamine as a valid alternative for HbA1c in the evaluation of glycemic control in generalized chronic periodontitis patients with type 2 diabetes mellitus. Further studies incorporating a larger sample size and including further variables are required before drawing more definitive conclusions and predictive models for periodontal disease.

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ANNEXURES

Annexure 1

INSTITUTIONAL ETHICAL COMMITTEE

Tamil Nadu Government Dental College and Hospital,Chennai.3
Telephone No: 044 2534 0343
Fax : 044 2530 0681

R.C No. 0427/DE/2010 Date: 18-03-2010.

Title of the work : A Comparative evaluation of correlation between periodontal disease status and plasma level of Glycated hemoglobin and fructosamine in patients with Generalised chronic periodontitis with type II Diabetes Mellitus.

Principal Investigator: Dr.H.Gayathri, IInd year MDS student,

Department : Dept. of Periodontics,
Tamil Nadu Govt. Dental College and Hospital,Chennai-3.

The request for an approval from the Institutional Ethical Committee (IEC) was considered for the following on the IEC meeting held on 18.01.2010 at the Principal's Chambers, Tamil Nadu Government Dental College & Hospital, Chennai-3.

"Advised to proceed with the study with change in the heading as the heading is quite long "

The Members of the Committee, the Secretary and the Chairman are pleased to approve the proposed work mentioned above, submitted by the Principal Investigator.

The Principal Investigator and their team are directed to adhere the guidelines given below:

1. You should get detailed informed consent from the patients/participants and maintain confidentiality.
2. You should carry out the work without detrimental to regular activities as well as without extra expenditure to the Institution or Government.
3. You should inform the IEC in case of any change of study procedure, site and investigation or guide.
4. You should not deviate from the area of work for which you have applied for ethical clearance.
5. You should inform the IEC immediately in case of any adverse events or serious adverse reactions. You should abide to the rules and regulations of the Institution.
6. You should complete the work within the specific period and if any extension of time is required, you should apply for permission again and do the work.
7. You should submit the summary of the work to the ethical committee on completion of the work.
8. You should not claim funds from the Institution while doing the work or on completion.
9. You should understand that the members of IEC have the right to monitor the work with prior intimation.
10. Your work should be carried out under the direct supervision of your Guide/Professor.

18/3/10

SECRETARY

18/3/10

CHAIRMAN

Annexure 2

INFORMED CONSENT FORM

AN EVALUATION OF THE RELATIONSHIP BETWEEN PERIODONTAL DISEASE STATUS AND GLYCEMIC CONTROL IN PATIENTS WITH TYPE 2 DIABETES MELLITUS

Name: O.P.No:

Address: Code No:

Age / Sex:

Tel. no:

I, _____, aged _____ years, am exercising my free power of choice and hereby voluntarily consent to be included as a participant in this study.

I agree to the following:

- I have been informed to my satisfaction about the purpose of the study and study procedures including the investigations to monitor and safeguard my body function.
- I understand that the lab investigations will require the procurement of my blood in required amount.
- I agree to cooperate fully and to inform my doctor immediately if I suffer any unusual symptom.
- I have informed the doctor about all medications I have taken in the recent past and those I am currently taking.
- I hereby give permission to use my medical records for research purpose. I am told that the investigating doctor and institution will keep my identity confidential.

Name of the patient Signature / Thumb impression

Name of the investigator Signature Date

Annexure 3

ஆராய்ச்சி ஒப்புதல் படிவம்

<u>ஆராய்ச்சி தலைப்பு</u>

"இரண்டாம் வகை நீரிழிவு நோயில், இரத்த சர்க்கரை அளவிற்கும் மற்றும் நாற்பட்ட பற்புறத்திசு நோயுக்கும் இடையேயுள்ள தொடர்பை ஆராய்ந்தறிதல்"

தேதி: நோயாளி எண்:
பெயர்: ஆராய்ச்சி சேர்க்கை எண்:
முகவரி: வயது: ஆ/பெ:
தொலைபேசி எண்:

நான் _____ வயது _____என்னுடைய சுயநினைவுடன் மற்றும் முழு சுதந்திரத்துடன் இந்த மருத்துவ ஆராய்ச்சியில் என்னை சேர்த்துக் கொள்ள சம்மதிக்கிறேன்.

எனக்கு விளக்கப்பட்ட விஷயங்களுக்கு நான் எனது சம்மதத்தை தருகிறேன்.

- இந்த ஆராய்ச்சியின் நோக்கம் மருந்துமுறைகள் பரிசோதனை முறைகள் எனக்கு திருப்தியுறும் வகையில் விளக்கப்பட்டன.

- நோயின் தன்மை அறியும் செயலின் ஒரு பகுதியாக என் உடலிலிருந்து சிறிதளவு இரத்தம் எடுக்கப்படும் எனவும், அது எந்த விதத்திலும் சிகிச்சையும் என் உடல் நலத்தையும் பாதிக்காது என்பதையும் அறிந்துக் கொண்டேன். மேலும், வழக்கமாக செய்யும் இரத்த பரிசோதனையும் இந்த ஆய்வுக்கு உபயோகப்படுத்தப்படுகிறது என்பதை உணர்ந்துக் கொண்டேன்.

- எனது மருத்துவ குறிப்பேடுகளை இந்த ஆராய்ச்சியில் பயன்படுத்திக் கொள்ள சம்மதிக்கிறேன். ஆராய்ச்சி மையமும் ஆராய்ச்சியாளரும் என்னுடைய பெயர் மற்றும் சில விவரங்களை இரகசியமாக வைப்பதாக அறிகின்றேன்.

பேராசிரியரின் கையொப்பம்	ஆராய்ச்சியாளரின் கையொப்பம்	நோயாளியின் கையொப்பம்

Annexure 4

DEPARTMENT OF PERIODONTICS
TAMILNADU GOVERNMENT DENTAL COLLEGE AND HOSPITAL,
CHENNAI 600003

An Evaluation of the Relationship between Periodontal Disease Status and Glycemic Control in Patients with Type II Diabetes Mellitus

PROFORMA

Name : Age / Sex:

O.P. No : Code No :

Occupation : Income :

Address and Contact No.:

Chief Complaints

Pain / Shaky teeth / Bleeding gums / Swollen Gums / Receding Gums / Pus Discharge / Increase in Spacing between teeth / Stains / Others.

Duration:

Medical history

1. Diabetes Mellitus.
    a. Duration : _____ years
    b. Recent change of anti-diabetic drug (past 4 months)
    c. Recent history of hospitalization with diabetic complication
2. Pregnancy / Lactation
3. Bleeding disorders or any other hematological disorder
4. Known systemic disease
5. Under steroids

Dental history

Periodontal treatment in the past 6 months

Clinical Examination

PLAQUE INDEX – SILNESS & LOE (1964)

17	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
47	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**

BLEEDING ON PROBING

Buccal													
17	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
Palatal / Lingual													
47	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**
Buccal													

CLINICAL ATTACHMENT LEVEL and PROBING POCKET DEPTH (in mm)

Buccal															
CAL															
PPD															
	17	**16**	**15**	**14**	**13**	**12**	**11**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	
PPD															
CAL															
Palatal / Lingual															
CAL															
PPD															
	47	**46**	**45**	**44**	**43**	**42**	**41**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	
PPD															
CAL															
Buccal															

DIAGNOSIS:

Sample Details

Date: **Time:**

INVESTIGATION:

Plasma HbA1c -

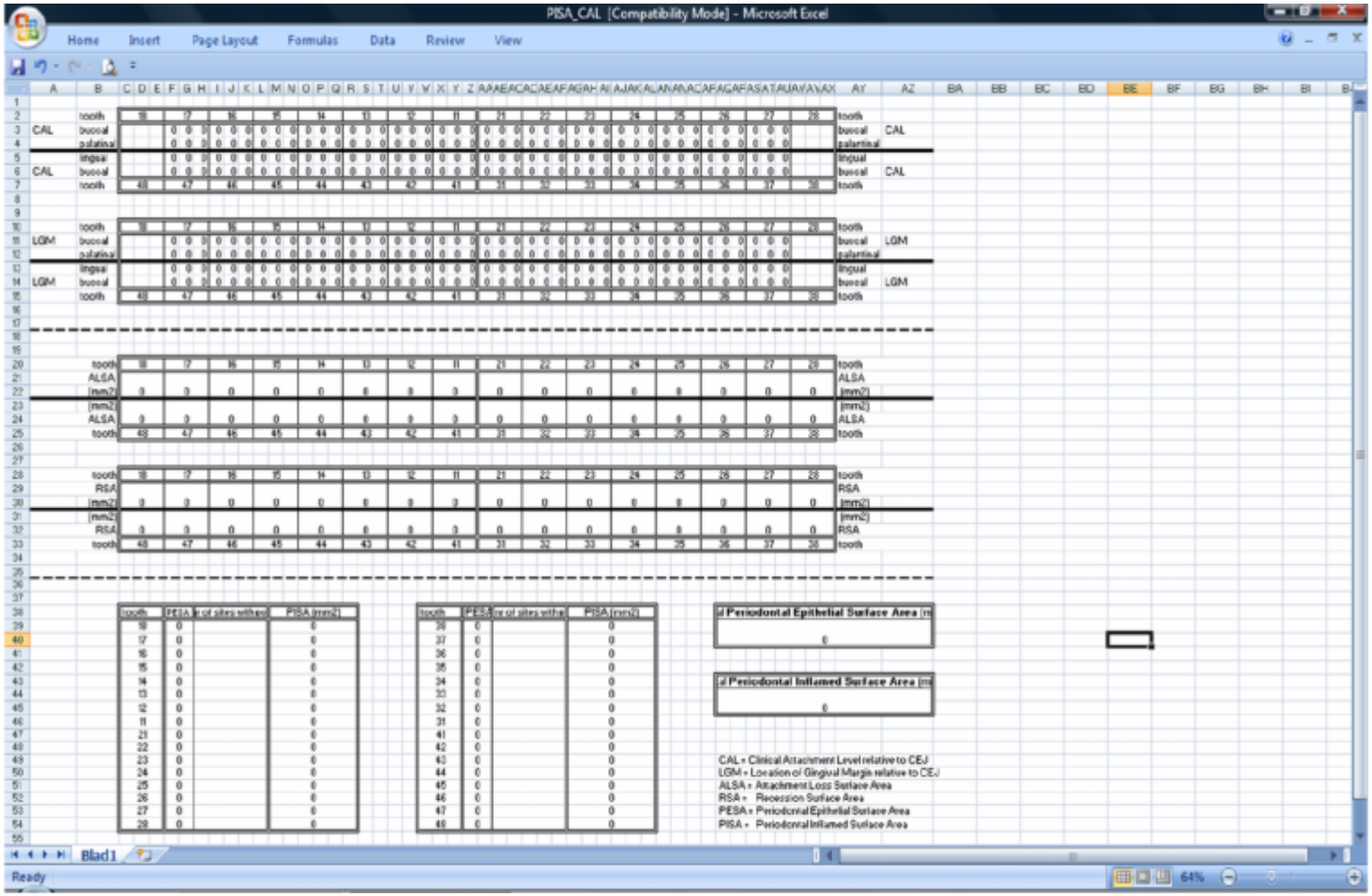
Serum Fructosamine -

INFERENCE:

Signature of the P.G student

Annexure 5

Excel spread sheet for calculation of ALSA and PISA



Annexure 6

Master chart

Diabetic group

Sl.No	Age	Gender	Duration	HbA1c	FA	Pl I	ALSA	PISA
1.	50	F	8	7.9	3.36	0.6	1618.99	375.87
2.	54	M	12	8.2	3.74	1.2	1846.16	488.68
3.	52	F	6	10.7	4.02	1.8	2311.18	619.59
4.	42	M	10	11.9	4.56	2.3	2558.84	1727.98
5.	42	F	4	10.1	3.65	0.7	1877.17	445.61
6.	48	F	5	8.9	3.6	0.78	1474.38	905.31
7.	55	F	8	9.6	4.37	0.65	1678.78	1091.23
8.	60	F	15	6.6	2.3	1.6	1648.29	285.24
9.	47	F	6	6.8	3.55	0.67	1601	366.06
10.	56	F	6	7.1	2	1.2	1848.74	395.81
11.	51	M	15	8.5	2.57	1.4	1739.01	460.5
12.	53	M	4	10.1	4.46	2.6	2627.46	1887.02
13.	35	F	7	6.4	2.45	1.32	1695.84	475.35
14.	55	F	15	8.7	2.95	2.32	1969.83	979.51
15.	59	F	9	10.2	4.88	1.3	2200.32	624.05
16.	48	F	7	9.2	3.83	2.25	1605.27	931.1
17.	54	M	5	10.6	4.69	2.04	2606.77	1431.95
18.	53	F	10	11.6	4.93	1.78	3031.1	1843.41
19.	55	M	8	11	4.12	1.45	2894.73	1752.2
20.	54	F	15	9.2	4	0.6	1329.93	312.95
21.	51	M	7	10.6	3.57	1.86	2494.8	1722.94
22.	50	F	4	11.2	5.08	1.32	2359.08	1698.88
23.	44	M	16	7	3.01	1.24	1111.92	479.38
24.	59	F	20	9.3	4.59	1.98	2994.08	1954.79
25.	56	F	7	8.7	2.69	2.45	3082.41	1766.35
26.	57	M	10	9.1	2.8	1.33	2089.22	1202.52
27.	58	M	8	10.2	4.9	2.76	3568.44	1385.2
28.	58	M	5	5.6	2.13	1.04	1007.86	219.15
29.	40	F	5	10.1	4.28	2.23	2358.23	1839.25
30.	38	F	7	7.9	3.25	2.34	1994.66	1046.96
31.	46	F	6	8.4	3.11	1.02	1467.22	424.33
32.	49	M	7	7.8	1.7	0.97	1681.76	337.9
33.	45	F	5	8.3	3.46	1.76	2383.8	896.79
34.	49	F	8	6.1	2.36	1.23	1863.65	348.17
35.	50	F	9	10.4	3.54	0.78	2001.12	1247.62
36.	56	F	5	9.1	4.21	0.67	2224.07	449.04
37.	58	F	4	8.1	3.52	0.78	1714.19	344.39

38.	51	M	4	9.5	4.04	1.04	2269.79	473.76
39.	51	M	8	10.5	5.14	0.7	2308.14	549.72
40.	45	M	6	6.9	2.94	1.06	1692.95	1211.11
41.	53	M	8	10.8	4.95	1.65	1919.34	1330.8
42.	50	M	12	8.4	3.56	0.65	1523.18	888.18
43.	57	M	10	10.4	4.55	1.28	2316.48	1118.8
44.	52	F	6	8.3	3.03	0.56	1796.92	399.98
45.	52	M	12	10.8	5.33	0.98	1857.7	1201.04
46.	57	F	10	8.2	3.8	1.23	1689.32	1142.08
47.	54	F	6	10.8	5.08	0.67	1937.61	637.94
48.	56	F	13	11	4.73	2.56	2305.61	1784.1
49.	58	F	15	10.9	4.66	1.02	2880.16	1640.78
50.	55	M	11	9.2	4.34	1.23	2696.84	1521.28
51.	48	F	6	8	2.63	0.52	1945.59	583.98
52.	47	F	5	10.5	4.92	1.78	2065.32	1325.52
53.	50	F	5	11.6	5.4	0.98	2391.23	828.5
54.	52	M	5	9.6	4.1	0.87	2234.69	1545.96
55.	48	F	8	10.4	4.57	0.68	2024.08	700.21
56.	49	F	10	8	3.24	0.75	1969.43	905.33
57.	56	M	3	9.6	3.89	1.45	2706.04	1207.01
58.	54	F	12	7.3	2.75	0.67	1897.7	482.49
59.	48	M	7	5.2	1.39	0.89	1592.06	1126.28
60.	49	F	6	7.7	2.78	1.65	1704.06	1489.21

Non-diabetic group

Sl.No	Age	Gender	HbA1c	FA	Pl I	ALSA	PISA
1.	48	0	5.2	1.98	0.97	1713.33	799.91
2.	35	1	5.1	2.12	1.23	2734.56	1598.29
3.	42	1	7.3	2.2	1.04	2203.99	1236.6
4.	59	0	4.8	2.16	1.08	1743.23	671.35
5.	58	0	5.8	2.22	1.3	2584.66	870.28
6.	46	0	5.2	1.94	1.08	2724.49	1680.42
7.	52	0	5.6	2.43	0.65	2469.41	1226.98
8.	44	1	6	2.19	1.02	2267.56	757.28
9.	40	0	5.2	2.16	1.12	2416.96	1207.56
10.	37	1	6.1	2.08	1.45	1559.39	943.72
11.	60	1	6.6	2.37	1.2	1401.57	837.53
12.	45	1	4.7	1.75	0.65	1778.13	884.74

13.	56	1	4.9	2.02	1.23	1799.91	400.21
14.	48	0	4.5	1.95	0.56	1188.93	426.15
15.	49	0	4.6	2.34	0.78	1405.25	471.62
16.	52	0	7.6	1.75	0.56	1957.02	1298.7
17.	56	1	7.4	2.11	1.23	1882.84	752.97
18.	53	1	4.9	1.8	1.21	2180.24	1136.74
19.	47	1	4.7	2.1	1.02	1644.84	619.07
20.	52	0	5	1.85	0.76	2651.32	1460.13
21.	60	0	5.5	2.33	1.32	1843.78	1133.28
22.	52	1	5.4	1.75	0.67	2337.58	482.28
23.	38	1	5	2.1	0.78	1223.5	438.26
24.	56	1	5.1	2.44	1.43	1972.56	1168.58
25.	47	1	5.3	2.6	1.56	2001.12	1293.05
26.	46	1	4.5	2	1.23	1527.37	459.99
27.	54	1	8	3.1	1.45	1782.2	1031.47
28.	49	0	5.9	2.12	1.76	2278.17	1260.92
29.	50	1	5.3	2.09	1.44	2391.23	1514.29
30.	57	1	7.5	3.17	2.13	1607.68	1162.86
31.	39	1	5	2.34	0.87	2314.83	1198.18
32.	46	0	5.6	2.43	1.56	4482.24	3276.44
33.	55	1	5.5	2.54	0.65	2309.95	824.24
34.	60	1	5.8	2.13	1.44	1723.81	1112.71
35.	48	1	7	4.59	1.65	2655.99	1966.29
36.	51	0	5.7	2.54	1.87	1741.68	1251
37.	49	0	5.4	3.44	1.54	1643.17	979.89
38.	40	1	5.4	2.64	1.76	3085.2	1858.05
39.	54	0	7.6	2.93	1.87	2358.23	892.83
40.	40	0	5.5	2.43	1.33	1723.42	1120.17